THE JOURNAL OF EXPERIMENTAL MEDICINE

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111548

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VOLUME FIFTH

WITH THIRTY-EIGHT PLATES AND EIGHTEEN FIGURES IN THE TEXT

NEW YORK
D. APPLETON AND COMPANY
LONDON: 33 BEDFORD STREET, COVENT GARDEN
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PROLIFERATION AND PHAGOCYTOSIS.

By F. B. MALLORY, M. D.

(*From the Scars Pathological Laboratory, Harvard University Medical School.*)

The effects which injurious agents, especially the toxines secreted by bacteria, produce on tissues are manifested in four different ways:

1, by degeneration or necrosis of cells;

2, by exudation from the blood-vessels;

3, by proliferation of cells; and

4, by phagocytosis, this term being used here to mean the inclusion and digestion of certain cells by other cells.

These four processes may occur separately, or in various combinations and proportions. The first two processes are generally accepted and taught. It is with reference to the last two, proliferation and phagocytosis, that I wish to present a certain amount of evidence bearing on the character of the cells concerned, on the nature of the toxines which excite the processes, on the duration and termination of the lesions, and on the relation of the processes to repair.

The view that toxines can cause various cells to proliferate, and certain cells to become phagocytic for other cells, was first forcibly called to my attention by a histological study of the lesions of typhoid fever published in this JOURNAL in 1898.[1] Since then I have studied these processes in various diseases in nearly a thousand autopsies occurring at the Boston City Hospital during the past three years.

What I wish to show is that strong toxines cause degeneration or necrosis of cells, and exudation, while dilute and weak toxines produce proliferation and phagocytosis. In support of this hypothesis I will cite in part the result of the study of large numbers of cases of certain diseases, in part a limited number of typical and of unusual lesions.

[1] *Journal of Experimental Medicine*, 1898, iii, p. 611.

Certain organisms, such as Staphylococcus pyogenes aureus and Bacillus diphtheriæ, produce strong toxines which act quickly, and chiefly locally: the effect on the tissues is to produce necrosis and exudation. Other organisms, such as Bacillus typhosus, produce mild toxines which act slowly, and cause proliferation or phagocytosis or both.

The stronger toxines, however, can produce these same processes of proliferation and phagocytosis when sufficiently dilute. The best example of this which I have found was in the kidneys of a girl five years old who died in four days with an acute myositis of the right thigh, due to Staphylococcus pyogenes aureus. The autopsy was made one hour after death. The femur was not affected. There were multiple miliary abscesses in the lungs, heart, and kidneys. In the kidneys they occurred for the most part in the pyramids, especially towards the bases. Very few of the foci were softened: most of them consisted of areas of necrosis with more or less infiltration with polynuclear leukocytes. Judged by inoculation experiments in rabbits, the lesions were not over 48 hours old.

Many of the lymphatics adjoining these beginning abscesses and running towards the bases of the pyramids were dilated and filled with numerous large phagocytic cells, together with a few lymphoid and plasma cells, and polynuclear leukocytes. The inclusions in the phagocytic cells consisted principally of polynuclear leukocytes and lymphoid cells. Careful study of these lymphatics showed occasional mitosis of the lining endothelium, and much more frequently migration of the endothelial cells through the walls into the adjoining connective tissue. The endothelial cells pass through the wall of a lymphatic in exactly the same way that the polynuclear leukocytes do; namely, a process of the nucleus surrounded by a minimum of protoplasm pierces the wall first, and the rest of the nucleus and the protoplasm gradually follow. After the endothelial cells contained inclusions they still showed the changes of form characteristic of amœboid motion, but none was found in the act of emigration.

In diphtheria the chief lesions are focal and occur for the most part in the air passages, in the immediate vicinity of the bacilli of diph-

theria; but there are other lesions due to the diffusion of a small amount of the toxine through the blood and lymph circulations. The most interesting of the latter are found in the lymph nodules of the tonsils, spleen, and gastro-intestinal tract. They consist of a moderate proliferation of the cells lining the reticulum. This proliferation usually occurs in the centres of the lymph nodules, but sometimes is diffuse or eccentric. Even more marked than the proliferation is the incorporation of the lymphoid cells of the lymph nodules by the cells lining the reticulum and by the new cells formed from them. Phagocytosis like proliferation usually begins in the centres of the lymph nodules and spreads peripherally, but may occur diffusely. In advanced lesions all or nearly all of the lymphoid cells in a lymph nodule may be destroyed. In such cases the centre of a lymph nodule shows a clump of epithelioid cells with the protoplasm more or less fused and containing no inclusions because all of the incorporated cells have been digested, and others are not within reach. Towards the periphery phagocytic cells containing partially digested cells are found, while at the periphery the phagocytic cells are stuffed with lymphoid cells still in a fair state of preservation. The large cells in the centres of the lymph nodules sometimes undergo necrosis, probably in consequence of lack of nutrition.

This brief statement of the proliferation and phagocytosis occurring in diphtheria is based on the histological study of 220 cases of diphtheria, of which a full report will be published shortly by Councilman, Pearce, and myself.

Pratt[2] has recently shown in a study of 50 cases of acute lobar pneumonia that Micrococcus lanceolatus causes marked proliferation of the cells lining the alveoli, the pleural cavities, and the lymphatics: these newly-formed cells are very phagocytic, incorporating and digesting polynuclear leukocytes, lymphoid and plasma cells, and red blood-globules. The proliferation is more marked in the early and in the late stages of the disease than in the middle stage when the exudation of polynuclear leukocytes is abundant.

[2] Contributions to the Science of Medicine dedicated by his Pupils to William Henry Welch on the Twenty-fifth Anniversary of his Doctorate, p. 265. Baltimore, 1900.

The acute lesions of the glomeruli of the kidney throw much light on the question of proliferation due to bacterial toxines. The organisms most frequently concerned are Micrococcus lanceolatus and Streptococcus pyogenes. The primary lesion from which the toxine escapes into the circulation is often an acute endocarditis; or the toxine may be secreted directly in the circulating blood in consequence of a septicæmia only. In other cases the primary lesion and the source of the toxine are more remote from the circulation. The lesions produced in the glomeruli vary from necrosis and exudation to pure proliferation.

The toxine secreted by Micrococcus lanceolatus causes proliferation almost exclusively of the endothelial cells lining the capillaries of the glomeruli. In one case (No. 1178) of acute endocarditis, in which death occurred at the end of four weeks, the kidneys showed an essentially pure type of acute proliferative intra-capillary glomerulonephritis. Mitotic figures in the endothelial cells were numerous; repeatedly two were found in one section of a single glomerulus.

With Streptococcus pyogenes the lesion produced in the glomeruli varies greatly; sometimes it is entirely exudative; at other times there is marked proliferation of the capsular epithelium or of the capillary endothelium: proliferation and exudation are often combined in various proportions.

In one case (B. C. H., No. 98.67) of streptococcus endocarditis and septicæmia there was marked proliferation of the capsular epithelium and also in places of the epithelium at the beginning of the tubules; in one place two mitotic figures in the renal epithelium were found side by side. Many mitoses were found in the capsular epithelium. In some of the capsular spaces there were a little fibrin and a few polynuclear leukocytes. Occasionally phagocytic cells were found within the capsular space: the inclusions consisted of polynuclear leukocytes and red blood-globules.

Certain organisms, such as the typhoid and tubercle bacilli, produce mild toxines which act slowly and usually produce proliferation only, but under certain conditions, especially when the organisms are massed together in large numbers, the toxines may be concentrated and produce necrosis and a purulent exudation.

In typhoid fever a mild diffusible toxine is formed which causes great proliferation of the endothelial cells lining the lymphatics, and the reticulum of the lymphoid tissue of the intestine and the mesenteric lymph nodes; it also causes proliferation of the endothelial cells lining the blood-vessels of the intestine, liver, and spleen. All of these newly formed cells are extremely phagocytic and incorporate lymphoid and plasma cells, polynuclear leukocytes, and red blood-corpuscles. Phagocytic cells formed within the blood-vessels are often carried by the circulation to the liver when they give rise to miliary infarctions by blocking up the capillaries. In the spleen the phagocytic cells may occlude the blood sinuses, giving rise to necroses.

Occasionally, and almost invariably following an attack of typhoid fever, the typhoid bacillus finds suitable conditions for abundant growth. Under these circumstances it produces a marked local reaction, namely, necrosis and purulent exudation like the more virulent organisms already mentioned.

The tubercle bacillus produces a variety of lesions. The typical lesion is of course the miliary tubercle, a clump of epithelioid cells produced by proliferation from endothelial and connective tissue cells. In some situations, as in the lungs, epithelial cells may also take part in the proliferation. Giant cells may or may not be formed. Ordinarily these epithelioid cells show little evidence of phagocytosis, but in certain situations, such as in the meninges of the brain, and in the lymph sinuses of lymph nodes, there may occur a very extensive diffuse proliferation of the endothelial cells which distend the sinuses widely and show marked phagocytic properties; so that an early diffuse tuberculosis of a bronchial lymph node may very closely resemble a mesenteric lymph node of the early, hyperplastic stage of typhoid fever.

On the other hand, in caseous pneumonia, the exudation is often as marked as the proliferation; and in certain rare cases the tubercle bacillus acts like the pus organisms and produces a purulent exudation. In these cases the organisms are present in great numbers, and the toxine is probably much more concentrated than usual.

In the lesions thus far considered, proliferation of endothelial cells

has played a very important part. It can be caused apparently by a great variety of toxines; several examples have been given. This proliferation always takes place by mitosis; careful search of fresh, well preserved tissue has always shown enough typical mitotic figures to account for the newly formed cells. In all of the situations in which the endothelium proliferates, except the glomeruli, the newly formed cells are extremely phagocytic. They incorporate a certain definite group of cells, namely, polynuclear leukocytes, lymphoid and plasma cells, and red blood-globules; they never incorporate epithelial or other endothelial cells. The included cells seem normal at the time they are taken up; occasionally the lymphoid cells have been in mitosis. After inclusion for a time in the phagocytic cells they show more or less evidence of degeneration; the nucleus first stains intensely, then dissolves more or less irregularly, and finally disappears.

The kind of cells which a phagocyte incorporates, depends on its situation in the tissue; if it lies in lymphoid tissue, it takes up lymphoid cells; if in a blood sinus of the spleen, it encloses red blood-globules, often a dozen or more.

In acute proliferative intracapillary glomerulonephritis I have never been able to find any evidence of phagocytosis.

Phagocytic cells necessarily have the power of amœboid motion, otherwise they could not incorporate other cells. In one very perfectly preserved case, the first mentioned in this paper, the young endothelial cells formed by proliferation from the lining endothelium of the lymphatics were found actively emigrating from the vessels into the surrounding tissue.

Under certain circumstances bacterial toxines cause proliferation also of epithelial cells. This is shown most convincingly in cases of acute capsular glomerulonephritis; mitotic figures are easily demonstrable and are often comparatively numerous. Occasionally the more highly differentiated epithelial cells at the beginning of the renal tubules also show active proliferation. Rarely these new-formed cells in the capsular spaces contain inclusions, usually polynuclear leukocytes or red blood-globules. They probably would contain more inclusions if the proper cells were within reach. Proliferation of

epithelium also takes place in pneumonia in the alveoli and in the pleural cavities; mitotic figures as a rule are readily found. The newly formed epithelial cells are desquamated often in large numbers and seem as phagocytic as endothelial cells, which they resemble in every way. The best proof that epithelial cells are phagocytic is the fact that they often can be found containing cell inclusions while they still are attached to the walls of the alveoli.

It is to be noticed that the epithelial cells which have been demonstrated to proliferate and to become phagocytic under the action of bacterial toxines are all cells of a low, undifferentiated type; they are flat cells which resemble endothelial cells and probably perform much the same functions.

There are two situations in which I have found phagocytic cells where their origin is less easily determined.

In one kidney of a very young child (B. C. H., No. 98.233), who died of diphtheria complicated with focal pneumonia and suppuration of both middle ears, there were found several small grayish areas, the largest 4 mm. in diameter, running from the papillæ of the pyramids into the cortex. The areas were rather soft but not fluid; in places small hæmorrhages had taken place into them. Unfortunately no cultures were made from these small areas which microscopically showed an acute pyelonephritis. The tubules in the pyramids were dilated and filled chiefly with phagocytic cells; a few polynuclear leukocytes were present, but most of them were enclosed within the phagocytes. In the connective tissue between the tubules there were many phagocytic cells and some polynuclear leukocytes, lymphoid and plasma cells. In the cortex the areas consisted almost entirely of phagocytic cells lying in the tubules and in the intertubular tissue. In some places all evidence of tubules had disappeared and there existed large areas of phagocytic cells with here and there a few polynuclear leukocytes, and lymphoid and plasma cells betweeen them. In the pyramids many of the phagocytic cells contained large numbers of bacilli which in their morphology and staining reactions resembled in every respect the colon bacillus. The organisms also occurred to some extent outside of the cells; in the cortex they were much fewer in number and occurred mostly in clumps.

So far as could be determined from a study of the sections the phagocytic cells arose entirely in the intertubular tissue. Several of them were found immigrating into the tubules, having passed but part way through the basement membrane. They were also often present in numbers between the renal cells and the basement membrane, apparently having pushed the cells from the wall. In other instances they had distended the lumina of the tubules and flattened the epithelial cells.

The second case is more difficult. In the epididymis of an actively functioning testicle removed from a man 72 years old in consequence of hypertrophied prostate, there were found great numbers of perfectly formed spermatozoa and also numerous huge phagocytic cells incorporating and digesting the spermatozoa by the hundreds. These phagocytic cells cannot be polynuclear leukocytes because the latter are never phagocytic for other cells. They can be only epithelial or endothelial cells; the latter view seems the more probable because it is difficult to conceive of the rather highly differentiated epithelial cells lining the epididymis being transformed into phagocytic cells.

The question whether bacterial toxines can cause connective tissue cells to proliferate and to become phagocytic is not easy to demonstrate. The best evidence is furnished, perhaps, by tuberculosis, but it is not conclusive. It is easy to show that a good deal of connective-tissue reticulum is formed between the epithelioid cells in the periphery of a miliary tubercle and also outside of the tubercle, but that does not prove that the epithelioid cells have produced that reticulum and therefore necessarily are connective-tissue cells. Wherever lymphoid and plasma cells are collected in numbers, a reticulum always forms between them. In the more chronic forms of capsular glomerulonephritis, a connective-tissue reticulum extends in from the capsule and out from the glomerular tuft, and spreads gradually between the epithelial cells in places forming a sort of basement membrane on which they tend to arrange themselves. In this way the capsular space is often divided into a number of small gland-like cavities. In neither of these two cases is it possible for the connective-tissue reticulum to have been produced by the cells which lie in its meshes.

The reticulum seems to grow in between the cells because there is a physiological need of it.

It is not improbable that a good many of the phagocytic cells in the typhoid lesions of the intestine, especially in the muscular coat, are derived from connective-tissue cells; but it is not easy to demonstrate it, partly because endothelial cells occur practically everywhere, partly because the connective-tissue cell, away from the intercellular substance it produces, possesses no very definite characteristics.

In connection with the subject of this paper the following case (B. C. H., No. 98.252) is of interest. A woman 48 years old, with alcoholic history, died of hypertrophic cirrhosis after symptoms lasting about seven months. The inner surface of the bladder except at the neck was found everywhere studded with slightly elevated, flattened, grayish to yellowish-gray nodules of a rather translucent appearance, varying in size from 1 mm. to 2 cm. in diameter. Only occasionally were the edges of the nodules overhanging. They projected from 1 to 4 mm. above the surface, and seemed to lie wholly within the mucous membrane as they were freely movable over the underlying muscle tissue. Two or three of the nodules showed slight hæmorrhages. So far as could be made out the nodules were not subdivided into papillæ, *i. e.* they did not present a cauliflower appearance. The mucous membrane over the nodules was smooth and glistening.

The condition was diagnosticated at first as multiple papillomata of the bladder.

Microscopic examination of the nodules after fixation of the tissue in Zenker's fluid, three hours after death, showed that they were due to collections of large phagocytic cells in the lymph spaces of the upper part of the submucosa. The cells were always most abundant just beneath the epithelium; in the smaller nodules they were confined to this situation, but in the larger nodules they extended more or less deeply into the submucosa, but never so far as to reach the underlying muscle tissue. The capillaries and larger blood-vessels in the nodules were more or less congested, and the tissue around them showed marked infiltration with lymphoid and plasma cells.

There were also numerous lymphoid and plasma cells beneath the mucous membrane and around the vessels between the nodules. The epithelium of the mucous membrane was usually well preserved. Over the nodules it was much thinned, and in places was reduced to a single layer of cells or was even missing.

The cells in the nodules varied a good deal in size, but usually were very large, and round, polygonal, or irregular in shape; the protoplasm as a rule was homogeneous and stained rather deeply with eosin. The nuclei usually single but sometimes two in number, were vesicular in character, round, oval, or irregular in shape, and usually eccentrically situated.

The inclusions in these large cells were of two sorts, bacteria and cells. The bacteria were short rods, in general occurring in small closely aggregated clumps which often lay within vacuoles. The number of organisms in a clump varied from half a dozen to dozens. Some cells contained as many as 7 or 8 clumps of organisms. Other cells contained from a few to hundreds of these organisms scattered diffusely in the protoplasm. The bacteria were found almost entirely within the phagocytic cells; some of them stained sharply, others faintly; many were evidently dead and stained faintly or deeply with eosin. In the cells where the organisms seemed to be growing most rapidly they were usually shorter and plumper than in the cells which contained fewer of them. Occasionally short chains of 2 to 4 members were found. The Gram staining method gave negative results. It is impossible of course to say what the organism is. Morphologically and tinctorially it belongs to the colon-typhoid group.

In places, especially within the larger nodules, nearly every phagocytic cell contained organisms. In other places they were not found in more than one cell in twenty. They were always more numerous near the surface of the nodules than in the deeper portions.

The cells included in the phagocytes were chiefly polynuclear leukocytes and lymphoid cells. Occasionally red blood-globules were incorporated. Most of the inclusions were so far digested that their nature could not be made out. The number of inclusions in a cell varied greatly, but rarely exceeded four or five, although occasionally

there were over a dozen. As a rule the inclusions lay within vacuoles. Within the protoplasm of many of the phagocytic cells were small hyaline bodies of varying size, stained pink and lying in vacuoles. Evidently they were the remains of incorporated cells. Many of them resembled closely the inclusions in the cells of carcinoma.

The nature of these phagocytic cells is difficult to determine absolutely. They resemble in all respects the phagocytic cells found in the lesions of typhoid fever. In all probability they are endothelial cells; but it is difficult to exclude entirely an origin from connective-tissue cells. Where the phagocytic cells were numerous, very little evidence of connective tissue could be found. At the bases of the nodules single phagocytic cells were present in the submucosa lying apparently in spaces between the strands of fibrous tissue. The inclusions in this situation consisted chiefly of hyaline, often concentrically layered, refractive, spherical bodies. The cells often showed forms suggestive of amœboid motion. They seemed to lie in lymph spaces. Distinct lymphatic vessels were very few in number; they contained phagocytic, lymphoid, and plasma cells. In the mucous membrane covering the nodules, phagocytic cells were often found, sometimes in considerable numbers, between the epithelial cells. They probably had migrated to that situation from the underlying tissue. Occasionally in some of the nodules there were small collections of leukocytes, attracted, so far as could be made out, by degeneration of phagocytic cells.

It seems reasonable to conclude that these tumor-like nodules composed of phagocytic cells lying in the submucous tissue of the bladder are due to the action of the organisms found in such large numbers within many of the cells. Certainly the nodules have none of the characteristics of a true tumor formation.

So far I have considered the action of toxines on only three forms of cells. Their action on three other forms I will mention briefly.

The great increase in the number of the polynuclear leukocytes in the blood in infections with certain bacteria, especially the pyogenic organisms, is well known. The leukocytosis produced is of diagnostic

importance; so also is the increase in the number of eosinophiles in trichinosis.[3]

Of greater significance in this connection is the increase of lymphoid and plasma cells which takes place in certain cases of scarlet fever and diphtheria. This has been considered at length by Councilman in his paper on Acute Interstitial Nephritis.[4] The cells collect in the veins of the pyramids in the kidney; they emigrate from the vessels into the intertubular tissue and continue to proliferate there; by their numbers they interfere with the function of the kidney and may cause destruction of the tubules. The process in some respects closely resembles a malignant growth, such as leukæmia or lymphosarcoma.

The tendency of the cells which proliferate under the action of toxines is to degenerate and disappear as soon as the agent to which they owe their existence is destroyed or is neutralized. The best example is afforded, perhaps, by the resolution which takes place in the hyperplastic patches of Peyer and in the mesenteric lymph nodes in typhoid fever. The affected tissues quickly recover their normal appearance. In certain situations the results of the proliferation are more permanent and more disastrous. For example, the renal glomeruli may be destroyed partially or entirely by occlusion of the capillaries, or by the formation of fibrous tissue in the capsular spaces, and be converted into small contracted masses of hyaline connective tissue.

The processes of proliferation and phagocytosis which I have been describing as due directly to the action of toxines are regarded generally as reparative in nature. Similar processes certainly occur in repair. Is it possible to differentiate them?

In repair, cells proliferate for definite purposes—epithelium to cover denuded surfaces; connective tissue and lymph endothelial cells to replace losses of tissue and to remove foreign bodies, such as necrotic cells, fibrin, fat, myelin, etc.; blood endothelium to form new blood-vessels for the nourishment of the new tissue.

The cells, however, which proliferate under the direct action of

[3] Brown, Trichinosis, with especial reference to the increase of the eosinophilic cells in the blood and muscle. *Journal of Experimental Medicine*, 1898, iii, p. 315.

[4] *Journal of Experimental Medicine*, 1898, iii, p. 393.

toxines, multiply greatly in excess of need, and show a lack of definite purpose; they may exert some more or less beneficent action, such as possibly the production of antitoxines, but they can no more be regarded as reparative than the exudation called out by the toxines; both may work infinite harm. As we have seen in the cases cited, phagocytic cells may block up lymphatics and undergo necrosis; they may be carried to the liver as emboli, blocking up the capillaries and giving rise to focal necroses of liver cells; they may enclose and destroy the lymphoid cells in lymph nodules and then undergo necrosis themselves; they may occlude the veins in the spleen, giving rise to infarctions; they may occlude the capillaries of the glomeruli by growth within the vessels or by pressure from without; they may give rise to tumor-like formations. Lymphoid and plasma cells may multiply in the circulation, and may invade and multiply in the kidney interfering with its functions and causing its destruction. The phagocytic cells are phagocytic beyond all bounds of necessity and destroy great numbers of active, useful cells. These are all abnormal and to a certain degree malignant properties.

A CONTRIBUTION TO STAINING METHODS.

I. A DIFFERENTIAL STAIN FOR CONNECTIVE-TISSUE FIBRILLÆ AND RETICULUM.
II. CHLORIDE OF IRON HÆMATOXYLIN FOR NUCLEI AND FIBRIN.
III. PHOSPHOTUNGSTIC ACID HÆMATOXYLIN FOR NEUROGLIA FIBRES.

By F. B. MALLORY, M. D.

(*From the Scars Pathological Laboratory, Harvard University Medical School.*)

I. A Differential Stain for Connective-Tissue Fibrillæ and Reticulum.

The following method for staining connective tissue is simple and is believed to be better than any yet proposed for that purpose. It is not absolutely differential because, besides connective-tissue fibrillæ and reticulum, it also stains certain hyaline substances, but these latter usually are so different morphologically that confusion cannot arise. The method is also useful for the study of fibrin, smooth and striated muscle fibres, and amyloid. The manner of staining is in brief as follows:

1. Fix in corrosive sublimate solution or in Zenker's fluid.
2. Embed in celloidin or paraffin.
3. Stain sections in a $\frac{1}{20}$ to $\frac{1}{10}$ of a one per cent aqueous solution of acid fuchsin 1 to 3 minutes.
4. Wash in water.
5. Place in a 1 per cent aqueous solution of phosphomolybdic acid for 1 minute or longer (use platinum or glass needle).
6. Wash in two changes of water.
7. Stain in the following solution for 2 to 20 minutes or longer:

Aniline blue soluble in water (Grübler),	0.5
Orange G (Grübler)	2.0
Oxalic acid	2.0
Water	100.0

8. Wash in water.
9. Dehydrate in 95 per cent alcohol.

10. Blot on the slide and clear in xylol, or clear in oleum origani cretici.
11. Xylol balsam.

The fibrillæ and reticulum of connective tissue, amyloid, mucus, and certain other hyaline substances stain blue; nuclei, protoplasm, elastic fibres, axis cylinders, neuroglia fibres, and fibrin, red; red-blood globules and myelin sheaths, yellow. The various structures do not stain with equal intensity, so that certain ones are brought out with great sharpness. This is particularly true of the fibrillæ and reticulum of connective tissue, also of fibrin and smooth and striated muscle fibres.

If it is desired to bring out the connective tissue as sharply as possible, omit the staining with acid fuchsin. Then the nuclei and protoplasm stain yellow and the blue fibrillæ and reticulum stand out more prominently.

The method is satisfactory only after hardening in corrosive sublimate solution or in Zenker's fluid, although fair results can be obtained after fixation by the method I have recommended for neuroglia fibres,[1] but the latter procedure is best suited to the central nervous system, under which subject it will be mentioned later. With alcohol and other fixatives everything stains blue.

The oxalic acid makes the aniline blue stain more intensely and quickly than it otherwise would. The orange G tends to limit the blue to connective tissue. The phosphomolybdic acid has two functions: it intensifies and fixes the acid fuchsin in certain histological elements while removing it from the connective tissue; it slows the action of the aniline blue and prevents it from gradually staining everything else in addition to the connective tissue.

The time of staining varies somewhat according to the structures it is desired to render prominent, the character of the tissue, and the thickness of the sections. Paraffin sections will stand longer staining than thick celloidin sections.

Elastic fibres stain pale pink or yellow. Sometimes the elastic laminæ of arteries seem to stain blue, but this is because the connec-

[1] *Journal of Experimental Medicine*, 1897, ii, p. 532.

tive tissue closely applied to the plates of elastic tissue stains deeply and conceals them.

This method is also useful in the study of fibrin, and of smooth and striated muscle fibres because they are brought out in sharp contrast to the connective tissue, especially if the staining with acid fuchsin is prolonged a little or if the sections are washed out for a few minutes in alcohol, which removes aniline blue rather quickly but does not affect the acid fuchsin.

The stain brings out clearly the deposit of amyloid in the tissues, especially the liver, because amyloid stains a light blue and stands out with the greatest sharpness from the red protoplasm of the liver cells. In places, where they have not degenerated, the connective-tissue fibrillæ can be seen running through or along side of the amyloid material.

The mucus in epithelial cells stains blue, as do also renal casts and certain other hyaline substances.

The method can be used also for the study of the central nervous system. It may be used after fixation in corrosive sublimate solution or in Zenker's fluid as already directed, or better still with slight modifications after the method I have recommended for the fixation of neuroglia fibres, namely:

Fixation.

1. Fixation of thin pieces of nervous tissue (2 to 5 mm. thick) in a 4 per cent aqueous solution of formaldehyde (10 per cent formol) for at least 4 days.
2. Saturated aqueous solution of picric acid 4 days or more.
3. 5 per cent aqueous solution of bichromate of ammonium 4 days in the incubator (use an abundance of the solution and change at the end of 24 hours so as to avoid any chance of a precipitate).
4. Alcohol. Embed in celloidin.

Staining Method.

1. 1 per cent aqueous solution of acid fuchsin, 2 to 5 minutes.
2. Wash quickly in water.
3. 1 per cent aqueous solution of phosphomolybdic acid, 1 to 2 minutes.
4. Wash in two changes of water.

5. 1 to 3 minutes in the aniline blue and orange G solution.
6. Wash in water.
7. Dehydrate in 95 per cent alcohol.
8. Blot and clear with xylol, or use oleum origani cretici.
9. Xylol balsam.

Connective tissue blue; neuroglia fibres deep red; axis cylinders and ganglion cells a lighter red.

II. Chloride of Iron Hæmatoxylin for Nuclei and Fibrin.

The results which may be obtained by this method are equally quick and satisfactory after all of the usual fixing reagents, except, perhaps, formaldehyde.

Celloidin or paraffin can be employed for embedding.

1. Stain sections on the slide for 3 to 5 minutes in a 10 per cent aqueous solution of ferric chloride.
2. Drain and blot the sections; then pour over them a few drops of a freshly prepared 1 per cent aqueous solution of hæmatoxylin. If all of the hæmatoxylin is precipitated by the excess of the ferric chloride, pour off the solution and add a fresh supply. In 3 to 5 minutes the sections will be colored a dark bluish black.
3. Wash in water.
4. Decolorize and differentiate in a $\frac{1}{4}$ per cent aqueous solution of ferric chloride. The sections should be kept constantly moving in the solution. The differentiation will be complete in a few seconds to one or more minutes.
5. Wash in water.
6. Dehydrate in alcohol.
7. Clear in oleum origani cretici.
8. Xylol balsam.

In the above directions definite strengths have been assigned to the solutions, but they may vary greatly without affecting the result. The important point is to get the sections stained deeply, and then to decolorize slowly. The differentiation can be stopped at any moment by transferring the sections to water. Sometimes it is advisable to examine the sections under the microscope to see if enough color has been extracted.

The strength of the hæmatoxylin solution is unimportant; it is

simply necessary to have enough hæmatoxylin to combine with all of the iron in and on the section. The simplest way is to dissolve by the aid of heat a pinch of the crystals in a few cubic centimetres of water in a test-tube. A little experience will determine about how much is needed. If a solution of hæmatoxylin more than one or two days old is used, the color obtained is grayish-blue and not so bright.

This method gives a sharp, permanent, dark blue stain to nuclei; it also stains fibrin of a grayish to dark blue color; if the decolorization is not carried too far, the contractile elements of striated muscle are brought out very sharply. In Zenker preparations the red globules appear of a greenish-gray color. Connective tissue is tinted a pale yellow. The nucleus of Amœba coli stains sharply by this method.

III. Phosphotungstic Acid Hematoxylin for Neuroglia Fibres.

This method was published originally in the Journal of Experimental Medicine (1898, III, p. 611). At that time the phosphotungstic acid manufactured by Merck was not pure. It contained a trace of phosphomolybdic acid and also some oxidizing agent which ripened hæmatoxylin at once. The staining solution made up according to the directions then given but with the pure phosphotungstic acid now manufactured gives negative results because the hæmatoxylin will not ripen spontaneously even after standing for months. The solution can be ripened, however, at once by the methods advised for alum hæmatoxylin; the best results are obtained by using peroxide of hydrogen.

To render the stain somewhat sharper, and to prevent the myelin sheaths from taking a bluish tint when the time of staining is prolonged, it has been found advisable to treat the sections with permanganate of potassium followed by oxalic acid.

The tissue should be fixed by the formaldehyde, picric acid, and bichromate of ammonium method described under the connective-tissue stain.

Staining Method.

1. Place the sections in a ½ per cent aqueous solution of permanganate of potassium for 15 to 30 minutes.

2. Wash in water.
3. 1 per cent aqueous solution of oxalic acid 15 to 30 minutes.
4. Wash in 2 or 3 changes of water.
5. Stain in the following solution 12 to 24 hours or longer.

Hæmatoxylin	0.1
Water	80.0
10 per cent aqueous solution of phosphotungstic acid (Merck)	20.0
Peroxide of hydrogen (U. S. P.)	0.2

Dissolve the hæmatoxylin in a little water by the aid of heat and add it, after cooling, to the rest of the water and the acid. Then add the peroxide of hydrogen. The solution keeps perfectly and can be used repeatedly.

6. Wash quickly in water.
7. Dehydrate quickly in 95 per cent alcohol.
8. Oleum origani cretici.
9. Xylol balsam.

Nuclei, neuroglia fibres, and fibrin, stain blue; axis cylinders and ganglion cells, pale pink; connective tissue, deep pink.

The blue color is a little sensitive to strong light, and on prolonged exposure will fade to pink.

If a permanent, isolated stain of the neuroglia fibres is desired, place the sections, after staining as directed in the phosphotungstic acid hæmatoxylin and washing in water, in a 30 per cent alcoholic solution of ferric chloride for 5 to 20 minutes (rarely longer); then wash in water and dehydrate as before. The nuclei, neuroglia fibres, and fibrin stand out sharply of a clear blue color; everything else is decolorized or appears of a pale yellowish or gray tint. The results obtained by this last step are practically identical with those obtained by either of the modified fibrin stains, and the method has the decided advantage of being applicable to any number of sections at once. The method will be found to bring out rather sharply in the nerve fibres outside of the cord the funnel-shaped markings in the myelin sheaths.

Phosphotungstic acid hæmatoxylin will be found occasionally useful as a stain for ordinary tissues hardened in various fixatives. Two to twenty-hours usually are required for staining. Nuclei, fibrin, elastic fibres, and the contractile elements of striated muscle stain blue; the other tissue elements stain pink to red.

A STUDY OF THE NEUROFIBRILS[1] IN THE GANGLION CELLS OF THE CEREBRAL CORTEX.

By STEWART PATON, Johns Hopkins University.

Plate I.

As has often been pointed out, the use of the terms chromatic and achromatic to distinguish certain characteristics of the ganglion cell is misleading. The words are only applicable to describe the stained and unstained tracts in nerve cells which have been stained by the Nissl methylene-blue method. In order to avoid confusion in describing the morphological characteristics of the ganglion cells, it has seemed to the writer advisable to use terms which as far as possible admit of general application and do not have reference simply to the results given by one particular stain. For this reason Marinesco's classification of the constituents of the ganglion cell into (1) chromatic, (2) achromatic and (3) amorphous elements, is open to criticism. In the present state of our knowledge of the morphology of the ganglion cell it is not possible to give more than a tentative classification of the cellular constituents. The various elements of the nerve cell may be roughly grouped as follows: (1) cytoplasm, (2) Nissl bodies, (3) fibrils, (4) constituents whose morphological characteristics are not yet definitely known.

The achromatic tracts are made up of at least two constituents, the cytoplasm and a definite specific fibrillary substance which possesses morphological characteristics by which it can be easily differentiated from the surrounding protoplasm in which it is held. There is also another sense in which the use of the term achromatic is not exact. Under certain conditions the achromatic tracts exhibit marked chromophilic tendencies. This is often due to the fact that the fibrils

[1] In this paper the term neurofibril is used in a purely morphological sense to describe the fibrils which lie wholly or only partly within the ganglion cells of the cerebral cortex.

are capable of being stained by reagents for which under normal conditions they have little or no affinity. In view of the fact that the fibrils are now known to be an important and definite constituent of the ganglion cell and on account of the present confusion of terms in cytological nomenclature it has seemed best to make the foregoing suggestions in reference to the selection of terms.

Although it is often possible to stain the fibrils by various methods there are none that are satisfactory except the hæmatin or the gold stains of Apáthy and the as yet unpublished methods of Bethe. As Apáthy's methods are somewhat capricious when an attempt is made to stain the fibrils in the ganglion cells in the higher vertebrates, I have decided to publish a modified hæmatin method which often, but not always, gives excellent results. I hope others may be induced to try the method and be able to propose further modification in the technique which will make the method capable of more general application.

The sections represented in Plate I, Figs. 1 and 2, were both taken from the cerebral cortex of a fully developed pig. The tissue immediately after death was placed for 24 hours in a saturated solution of bichloride of mercury, to which 5% of glacial acetic acid had been previously added. Other methods of fixation can also be used. After the fixation the tissue is transferred to 95% alcohol. The alcohol must be changed twice during the first 24 hours and then every other day for a week. After this the fluid need not be changed oftener than once a week. In two weeks the sublimate is thoroughly removed from the tissues. I believe that better and more reliable results are obtained by not using the iodine solutions to remove the sublimate. This chemical has a very deleterious effect on the tissues. After being hardened in alcohol the tissue is ready for embedding. Either celloidin or paraffin may be used, preferably the latter, care being taken to use chloroform instead of xylol. The sections are fixed to the slide by the use of distilled water, and, after drying and the removal of the paraffin by chloroform, are put into 95% alcohol. The slide is then put for 1-2 hours into tinctura ferri Rademacheri as recommended by Weigert in his "mitosis stain." The sections are quickly rinsed in distilled water and are stained for 24 hours in the Apáthy[2] hæmatin solution. The

[2] *Mittheil. aus der zoolog. Station zu Neapel*, 1897, xii, Hft. 4.

whole section is then found to be over-stained. The preparations are then bleached under the microscope in an aniline oil and alcohol mixture (one part of the former to nine parts of 70% alcohol). The slides are finally well washed in tap-water, rinsed in distilled water, and after dehydrating and clearing are mounted in the usual way in Canada balsam dissolved in chloroform. The specimens when once fixed can be kept indefinitely.

As said already, the method is not yet entirely satisfactory. In the human nervous system the results are only very rarely good; but I believe this is due to the fact that it is so difficult to obtain perfectly fresh material. The ganglion cells in the human cerebral cortex when stained by this method, as a rule, give pictures exactly similar to those obtained when sections have been made from the brains of animals which have not been fixed immediately after death. In both cases the fibrils are only very imperfectly stained. This fact has an important bearing if there are those who still contend that the fibrils are artefacts, or are only the results of post-mortem changes. Arnold [3] has said recently that he was unable to find any long fibrils in the ganglion cell, but only short ones together with small granular masses. His observations were made on the ganglion cells in the human nervous system. The continuity of the fibrils after death is soon broken and there follows a period of granular degeneration. As a rule, I believe, the fibrils are very susceptible to post-mortem changes, and these changes are first seen in the body of the cell. Apparently the fibrils in the processes, particularly in the apical process of the pyramidal cells, remain for a considerable period after death unchanged. This may account for v. Lenhossék's statement in regard to the fibrillary substance. He admits the presence of the fibrils in the apical processes, but does not believe that the fibrils exist in the cell body. It can be very easily shown that the post-mortem degeneration of the fibrils begins in the cell body, and in specimens which are imperfectly fixed the cell body often has a granular appearance in strong contrast to the apical processes where the fibrils can be plainly seen.

In both sections from which the drawings for Plate I were made, it is possible to follow fibrils which run through nearly the whole

[3] *Archiv f. mikr. Anat. u. Entwick.*, 1898, lii, p. 535.

length of the cell. Similar fibrils have been described by Bethe. In sections thicker than those represented in the plate, fibrils can often be seen running through the whole length of the cell. As already stated, Arnold does not believe that any long fibrils are found in the ganglion cell, but only "short fibrils and granular masses." I am convinced that the failure of Arnold to find longer fibrils in the ganglion cell is due to the technique which he has employed. The use of strong iodine solutions makes the staining of the fibrils difficult and often impossible.

In comparing Figs. 1 and 2 certain important differences are recognizable. In the section represented in Fig. 1 the fibrils run straight through the cell processes without being connected with each other, but in the cell body there are connections between the individual fibres, so that a very wide-meshed network is formed. This agrees with the description given by Apáthy, and does not support Bethe's view. The latter failed to find any network formed within the cell body. In the apical process of the ganglion cell, shown in Fig. 2, it is easy to distinguish the fibrils, many of which can be followed through the whole length of the process and well into the cell body. In the preparation from which the drawing was made a bundle of fibrils can be followed from a point at about the outer fourth of the apical process through the remainder of the process into the cell body almost to its base, near where the axone is given off. Another bundle of fibrils can be followed which enter the ganglion cell from the ground substance on one side at about the level of the nucleus. This bundle passes inward and downward below the nucleus, and leaves the cell by one of the basal processes on the opposite side of the cell.

In the section from which the drawing for Fig. 2 was made it can easily be seen that the diffuse network of fibrils which is visible is not within, but is superimposed on, the cell and its processes. The fibrils here lie in a different and more superficial plane than that of the cell and its processes. I am convinced from my own observations that it is this extracellular network of fibrils which Held[4] has described as a pericellular network of terminal axones. The same is

[4] *Arch. f. Anat. u. Entwicklungsgesch.*, 1897, Suppl.-Bd., p. 273.

true also of the reticulum surrounding the nerve cell described by Golgi.[5] It is altogether impossible by means of the silver stains to differentiate between the fibrils and the processes of the cells, so that these methods are no longer of any service in studying the structure of the ganglion cells. By this modified hæmatin method it is possible to demonstrate the passage of the fibrils from the cell body, as well as from the processes, into the intercellular substance. The discussion of the relation of the neurofibrils to the intranuclear network as well as to the grey substance will not be considered in this paper.

After a careful study of the development of the ganglion cell[6] I have been brought to the conclusion that the presence or absence of "gemmules" is dependent upon the existence or non-existence of the fibrils. Hill[7] concluded that these lateral buds or gemmules were nothing more or less than "the cell end of an unstainable nerve filament." This is, I believe, only partly true. A gemmule is formed by a silver deposit at the point where the nerve filament or fibril enters the cell process, and it is also found if the fibril is simply lying upon the process as is the case with most of the fibrils in Fig. 2. The gemmules are found either at the point of entrance, or at the point of simple contact of a fibril with the protoplasm of the cell process. It can be easily shown that the appearance of the gemmules in the cortical cells of embryos is synchronous with the appearance of the fibrils. In the adult as well, the gemmules depend upon the existence of the fibrils and any pathological process which destroys the latter causes a corresponding diminution or absence of the former.

DESCRIPTION OF PLATE I.

Both sections are from the cerebral cortex of a fully developed pig. Thickness of sections 2μ. Zeiss, Apochromat. No. 6, Ocular, 2 mm.

FIG. 1. The neurofibrils can be seen running through the cell-body and apical process. In the section fibrils can be seen entering the ground-substance from the cell-body as well as from the processes.

FIG. 2. Most of the fibrils, which look as if they were within the cell-body, are really extracellular, and form part of the fibre-network of the ground-substances.

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[5] *Arch. ital. de biol.*, 1898, xxx, p. 71.

[6] The histogenesis of the cellular elements of the cerebral cortex. Contributions to the Science of Medicine dedicated by his Pupils to William Henry Welch on the Twenty-fifth Anniversary of his Doctorate, p. 709. Baltimore, 1900.

[7] *Brain*, London, 1897, xx, p. 131.

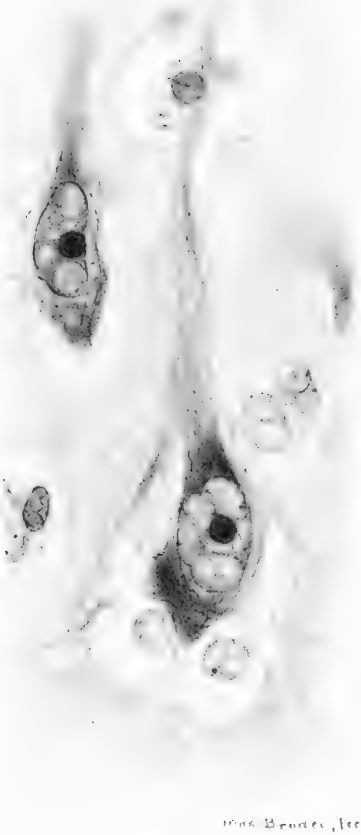


FIG. 1.

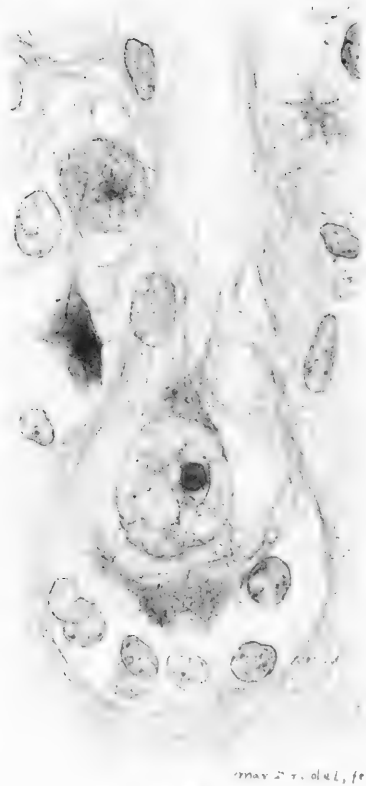


FIG. 2.

AN EXPERIMENTAL STUDY OF OXALURIA, WITH SPECIAL REFERENCE TO ITS FERMENTATIVE ORIGIN.

By HELEN BALDWIN, M. D.

(*From the Laboratory of Dr. C. A. Herter, New York City.*)

After the discovery of calcium oxalate crystals in the urine by Donné[1] in 1838, there followed a careful study of the symptoms associated with their deposit. This resulted in the description of a so-called oxalic-acid diathesis, which was thought by certain clinicians, notably Prout,[2] Golding Bird,[3] and Begbie,[4] to be of considerable importance and interest. Smoler,[5] however, found that the crystals were present in fifty-two per cent of four hundred specimens of urine, and Bacon, in forty-one per cent of nine hundred and nine cases, from patients suffering from almost every form of disease, and Neubauer[6] showed that calcium oxalate was almost constantly present in solution in the urine, even when not precipitated. These discoveries led to the belief that oxaluria is a symptom of many diseases, and not in itself of grave import. In 1896, Dunlop[7] published an article which supported the view that all the oxalic acid excreted in the urine had been ingested with the food, and that it was never formed in the animal organism by metabolism.

I have made the following series of observations and experiments: (1) to test the accuracy of Dunlop's conclusion; that is, to determine whether oxalic acid is ever formed in the animal body; (2) to study the influence of the ingestion of oxalic acid in foods upon the amount

[1] Donné, *Compt. rend. Acad. d. Sc.*, Paris, 1839.

[2] Prout, On the Nature and Treatment of Stomach and Urinary Diseases. London, 1840.

[3] Golding Bird, Observations on Urinary Concretions and Deposits. London, 1842.

[4] Begbie, *Monthly Journ. Med. Sc.*, Edinburgh and London, 1848–9, ix, p. 943.

[5] Smoler, *Prager Vrtjschr. f. d. prakt. Heilk.*, 1861, lxix, p. 157; lxx, p. 35.

[6] Neubauer, *Arch. d. Vereins f. gemeinsch. Arb. z. Förd. d. wiss. Heilk.*, 1860, iv, p. 1. Neubauer und Vogel, Analyse d. Harns. Wiesbaden, 1863.

[7] Dunlop, *Journ. Pathol. and Bacteriol.*, 1896, iii, p. 389.

excreted in the urine; (3) to study the physiological action of soluble oxalates with a view to deciding in what measure the presence of oxalic acid in the system is responsible for the symptoms attributed to the "oxalic-acid diathesis."

Occurrence of Oxalic Acid in Foods.—Oxalic acid and its salts are found widely distributed in nature. They are present in many of the common forms of vegetables, grains and fruits. Esbach [8] and Auerbach [9] have made estimations of the oxalic acid in the common foods. Some of those which they found to be rich in oxalic acid are spinach, rhubarb, dried figs, cocoa, tea, coffee, pepper, potatoes, beetroot, green beans, plums, tomatoes and strawberries. Foods which they found to contain little or no oxalic acid are peas, asparagus, cucumbers, mushrooms, onions, lettuce, rice, cauliflower, pears, peaches, grapes, melons, and wheat, rye, and oat flour.

In connection with the following experiments, a few foods were examined in order to select a diet free from oxalates, which should be more liberal than Dunlop's exclusively milk diet. The list of foods free from oxalic acid includes proteids (meat, milk and eggs) with sugar, butter, corn meal, rice, and the Huntley and Palmer breakfast biscuits.[10]

Characteristics of Calcium Oxalate Crystals.—When calcium oxalate is rapidly precipitated from a solution it falls in a very finely divided crystalline deposit, appearing like an amorphous sediment. These crystals contain only one molecule of water of crystallization. If allowed to stand a number of days, these crystals may group themselves in dendritic or star forms, similar to certain of the phosphatic crystals. After standing from ten days to three weeks, the characteristic octahedral crystals frequently form. These contain three molecules of water of crystallization.

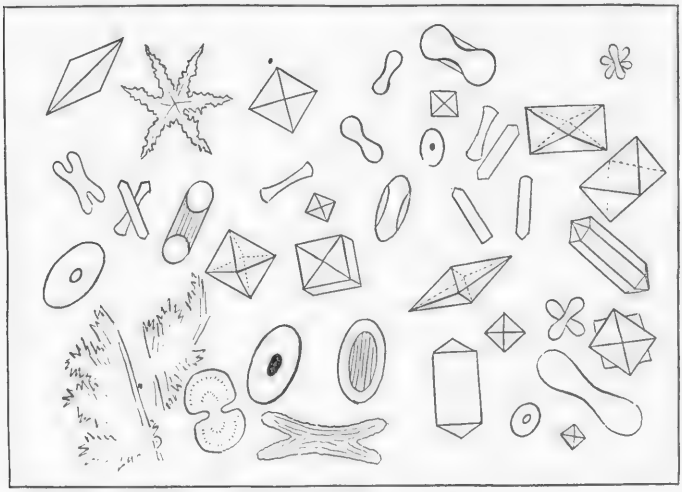
In the urine, calcium oxalate usually forms in octahedra, but it may

[8] Esbach, *Bull. gén. de thérap.*, Paris, 1883, civ, p. 385.

[9] Auerbach, Virchow's *Archiv*, 1879, lxxvii, p. 226.

[10] The food tested was cut or ground in small pieces, boiled in dilute hydrochloric acid, allowed to stand forty-eight hours, filtered and washed free from acid. The filtrate was then neutralized with ammonia, rendered very slightly acid with acetic acid, and then treated like the urine by a method described below.

be found in a variety of crystalline forms, described by Fürbringer[11] as following two types, the prismatic and spheroidal (see the accompanying figure). These crystals which are clear and colorless are insoluble in ammonia and alcohol, almost insoluble in hot and cold water (1: 500,000 (Storer) = 2 mg. per litre), slightly soluble in acetic acid (3-9 mg. in 20-60 cc. dilute acetic acid (Nickel[12]), but are readily dissolved by the strong mineral acids.[13]



Varieties of Calcium Oxalate Crystals.

Quantitative Estimation of the Calcium Oxalate in the Urine.— The quantitative estimation of the calcium oxalate found in the urine is a long and tedious process, and, unless great care is used, it is liable to large percentages of error. The method employed in these experi-

[11] Fürbringer, *Deutsch. Arch. f. klin. Med.*, 1875, xvi, p. 519.

[12] Nickel, *Ztschr. f. physiol. Chemie*, 1887, xi, p. 186.

[13] The solubility of calcium oxalate as tested in this series of experiments was found to be as follows:

1. In cold distilled water 2.2 mg. per litre.
2. In boiling water 1 mg. per litre.
3. In 2.5% acetic acid, after standing 48 hrs., 28 mg. per litre.
4. By washing with 2.5% acetic acid, 8.2 mg. per litre.

ments is founded on that of Dunlop.[14] It differs essentially from the older methods of Neubauer[15] and of Shultzen[16] in that the calcium oxalate is precipitated from an acid solution by means of alcohol, instead of from an alkaline solution by calcium chloride or calcium hydrate.

Dunlop's method (slightly modified). The urine should be thymolized as soon as passed to prevent fermentation and the precipitation of phosphates. If the specimen is alkaline, render it slightly acid with acetic acid.

To 500 cc. of a well mixed specimen of the twenty-four hours' urine, add 150 cc. of over 90% alcohol, to precipitate the calcium oxalate. Set aside for forty-eight hours. Filter, washing the beaker carefully, and removing crystals from the sides by rubbing with a rod protected by rubber tubing. Wash the sediment thoroughly with hot and cold water and with dilute acetic acid (1%). Place the filter in a small beaker and soak in a small amount of dilute hydrochloric acid. Then wash with hot water till there is no further acid reaction; filter the washings and evaporate the filtrate to about 20 cc. Add a very little calcium chloride solution to ensure an excess of calcium; neutralize the hydrochloric acid with ammonia, and then render the solution slightly acid with acetic acid. Add strong alcohol to the amount of 50% of the volume of the fluid and set aside forty-eight hours. Collect the sediment on an ash-free filter, wash with cold water and dilute acetic acid till free from chlorides. (Avoid the use of hot water, as it carries the finely divided precipitate through the pores of the filter). Incinerate first over a Bunsen burner and afterwards for five minutes in a blowpipe flame, cool over sulphuric acid, and weigh. The ash is calcium oxide, each gramme of which represents 1.6 grms. oxalic acid.

In the older methods of Neubauer and of Shultzen, the urine was rendered alkaline, and the oxalic acid precipitated by adding an excess of calcium chloride solution. In this way the phosphates, as well as calcium sulphate, are precipitated, and can with difficulty be separated from calcium oxalate; for, unless large quantities of water and acetic acid are used in washing, traces of phosphates and sulphates remain and are weighed in the ash, whereas, if enough of these is used to dissolve away the impurities, there is a weighable amount of calcium oxalate dissolved and lost. In using the method either of Neubauer, Shultzen, or Dunlop, the ash should each time be tested for phosphates and sulphates.

The following experiments (Table I) were made to test the accuracy of Dunlop's method; 500 cc. of a well mixed twenty-four hours' specimen of urine was examined for the amount of oxalic acid present and the result noted. Then to another 500 cc. of the same specimen was added a weighed amount of calcium oxalate and the mixture treated as in the first instance. When using the utmost care, the loss will be about one milligramme.

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[14] Dunlop, op. cit.

[15] Neubauer and Vogel (Huppert), Analyse d. Harns. Wiesbaden, 1898. (Fürbringer and Czapek's modification of Neubauer's method.)

[16] Shultzen, *Arch. f. Anat. u. Physiol.*, 1868, vi, p. 719.

TABLE I.

EXPERIMENTS TO TEST THE ACCURACY OF DUNLOP'S METHOD.

	Calcium oxalate added to urine.	Calcium oxalate recovered.	Calcium oxalate lost.
1.....	.0289 grm.	.0270 grm.	.0019 grm.
2.....	.0169	.0158	.0011
3.....	.0232	.0256	Trace of phosphates in ash.
4.....	.0203	.0201	.0002 grm.
5.....	.0150	.0150	.....
6.....	.0281	.0282	.....
7.....	.0202	.0208	Faint trace of phosphates.
8.....	.0281	.0280	.0001 grm.
9.....	.0453	.0455	Faint trace of phosphates.
10.....	.0289	.0270	.0019 grm.

Salkowski[17] has described a method of separating the phosphates from the oxalates by shaking out with ether. This does not seem necessary in the case of human urine when Dunlop's method is used. But in dog's urine, with high specific gravity, it is very difficult to remove the phosphates even while using Dunlop's method.

Oxalic Acid in Normal Urine.—In health the amount of oxalic acid excreted in twenty-four hours varies with the amount ingested in the food. The average is estimated by Fübringer as .02 grm. The following tables show varying amounts excreted with different diets:

TABLE II.

NORMAL URINE. DIET CHIEFLY CARBOHYDRATES. (MEAT, MILK, BREAD, POTATO, OATMEAL.)

Vol.	Sp. gr.	Oxalic acid in 24 hours.	Calcium Oxalate crystals in sediment.
1305	1017	.0058	None.
1235	1020	.0004	Few.
1615	1015	.017	Numerous.
1295	1012	.013	None.

TABLE III.

NORMAL URINE. FOOD RICH IN OXALATES. MIXED DIET WITH LARGE AMOUNTS OF TOMATO, SPINACH, TEA AND COFFEE.

Vol.	Sp. gr.	Oxalic acid in 24 hours.	Calcium Oxalate crystals in sediment.
1765	1011	.0011	Present.
1865	1017	.0012	"
1945	1015	.0006	"
1760	1016	.0028	"

[17] Salkowski, *Centralbl. f. d. med. Wiss.*, 1899, xxxvii, p. 257.

TABLE IV.

NORMAL URINE. MIXED DIET.

Diet.	Vol.	Sp. gr.	Oxalic acid in 24 hrs.	Oxalic acid per litre.	Calcium oxalate crystals in sediment.	Impurities in ash.
Ordinary mixed diet	1645	1016	.0047	.0029	None.	
"	1360	1018	.0022	.0016	"	
"	1185	1014	.0099	.0083	"	
"	1065	1019	.002	.0019	"	
"	750	1028	.0017	.0014	Numerous.	
"	865	1026	.0147	.0170	None.	Trace of phosphates.
"	730	....	.015	.021	Few.	"
" + rhubarb	480	1034	.034	.072	Large, numerous.	Trace of phosphates and sulphates.
Ordinary mixed diet	800	1022	.024	.030	Numerous.	"
"	1720	1010	.011	.006	Small.	
"	720	1022	.009	.0128	None.	
"	1115	1019	.017	.015	Numerous.	Trace of phosphates.
"	1210	1014	.0035	.0014	None.	
"	1205	1013	.0017	.0016	Few.	
"	950	1019	.0076	.008	"	
"	820	1022	.0103	.0116	Very few.	
"	950	1021	.0016	.0006	" "	
"	950	1024	.0115	.012		
"	1225	1014	.0047	.0038		
"	1250	1013	.0169	.0135		
"	1225	1013	.0031	.0026		
"	1300	1013	.0062	.0048		
"	2000	1012	.0062	.0031		
"	1700	1012	.0011	.0006		

There were also examined thirty-five specimens from patients who were receiving a mixed diet. These patients were suffering from gastric and intestinal disorders. The urinary examinations gave the same results as in the case of the healthy persons. The daily excretion of oxalic acid varied from a few milligrammes to about two centigrammes, the mean falling below ten milligrammes. It was also noted, as Fübringer had shown, that the precipitation of calcium oxalate crystals seemed to bear no relation to the amount in solution in the urine.

Characteristics of Urines Precipitating Oxalate of Lime.—The conditions causing precipitation of calcium oxalate in the urine are

not known. The characteristics of the urines of oxaluria have been studied by Begbie, Golding Bird, Maclagan and others. Fürbringer has shown that the crystals may be absent when a specimen contains a large amount of oxalic acid or present when there is only a trace. They are found with every degree of acidity, from highly acid to alkaline, with every degree of specific gravity and with every color. The following observations are collected from Dr. Herter's records.

In 370 cases in which the sediment was examined calcium oxalate crystals were found in 94, that is 25.4 per cent. The acidity varied from .091 grm. oxalic acid to alkaline, the mean acidity was .012 grm. The color varied from 1 to 7—mean 4—(Vogel's Chart). Thirty per cent of the specimens contained uric acid or urates in the sediment. The ratio of urea to uric acid varied from 23.4 to 99.2, the mean being 43.5. The lowest specific gravity was 1014, the highest 1035, the mean 1026. To determine whether the concentration of the urine affected the precipitation of calcium oxalate, five specimens of urine containing octahedral crystals were filtered and the filtrates concentrated at a low temperature until the specific gravity reached from 1032 to 1037. In no case was there a further precipitation of oxalate crystals.

ON THE FORMATION OF OXALIC ACID IN THE ANIMAL ORGANISM.

The origin and significance of the oxalic acid found in the urine, has formed the subject of much discussion. Since the observations of Prout, it has been recognized that the oxalic acid taken in the food or in drugs may in part reappear unchanged in the urine. Dunlop claims that all the oxalic acid found in the urine is taken into the body in this way. He bases his opinion on the fact that in patients placed on a milk diet, he was unable to recover any oxalic acid from the urine by quantitative analysis. In accordance with this, Gaglio,[18] Bunge,[19] and Burggraeve,[20] feeding dogs on meat alone, found no oxalic acid in the urine. But opposed to Dunlop's hypothesis are the experiments of Salkowski and of Auerbach,[21] who found oxalic acid in the

[18] Gaglio, *Arch. f. exp. Path. u. Pharm.*, 1887, xxii, p. 235.
[19] Bunge, Lehrb. d. phys. u. path. Chemie. Leipzig, 1889.
[20] Burggraeve, *Bull. Acad. roy. de med. de Belg.*, 1862, 2 s., v, p. 327.
[21] Auerbach, Virchow's *Archiv*, 1879, lxxvii, p. 226.

urine of fasting dogs, and those of Wesley Mills,[22] who found oxalic acid in the urine of dogs on meat diet. Primavera has reported an instance of a diabetic patient, with oxaluria, who, when placed on a meat diet, still had a heavy deposit of calcium oxalate in the urine. Bearing on this point, the experiments recorded in Tables V, VI and VII were made:

TABLE V.

PATIENTS ON DIET FREE FROM OXALATES.

	Date.	Diet.	Amount of oxalic acid in 24 hours.	Remarks.
Case I		Continuous milk diet, beginning Nov. 10.		
"	Nov. 16	Milk.	None.	
"	Nov. 17	"	"	
"	Nov. 18	"	"	
"	Nov. 19	"	Slight trace.	
"	Nov. 20	Milk and sugar.	None.	
"	Nov. 21	Milk and sugar, 45 grms.	None.	Sediment examined after precipitation by alcohol. No calcium oxalate crystals.
"	Nov. 22	Milk and sugar, 45 grms.	"	Calcium oxalate crystals formed.
"		Mixed diet from Nov. 23–Nov. 30.		
"		Milk diet Dec. 1–Dec. 4.		
"		Milk and sugar, 45 grms. Dec. 5–14.		
"	Dec. 8	Milk and sugar.	3.9 mg.	No calcium oxalate crystals.
"	Dec. 14	" "	1.1 mg.	Calcium oxalate crystals formed.
Case II....		Milk.	None.	
"	Nov. 3	"	"	
"	Nov. 4	"	"	
Case III ..		Milk, 1 week.	"	
Case IV ..		Meat, 1 year.	Slight trace.	
Case V ..		Milk and beeftea.	None.	
Case VI ..		Milk.	None.	
Case VII ..		Milk, 4 days.	Trace.	
Case VIII..	June 24	Carbohydrate diet free from oxalates for 1 week.	3.4 mg.	Calcium oxalate crystals rather numerous. Ash free from phosphates and sulphates.
" ..	June 25	Same.	25.5 mg.	Same as above.
Case IX ..	June 30	Same as above.	37.4 mg.	Calcium oxalate crystals large and numerous. Phosphates in ash.
" ..	July 1	" "	25. mg.	Calcium oxalate crystals large and numerous. No impurities in ash.

[22] Wesley Mills, *Journ. of Physiology*, 1885, v, p. 231.

TABLE VI.

DOG'S URINE. DIET FREE FROM OXALATES.

Diet.	Vol.	Amount of oxalic acid.	Sediment examined after precipitation with alcohol.
Meat	210 cc.	0	
"	100	0	
"	500	0	
"	180	0	
Glucose, 5 grm. Cornstarch, 5 grm. Water, 300 cc.	250	.004 grm.	Calcium oxalate crystals.
Cornstarch, glucose	285	.0012 grm.	
Meat	550	0	
"	170	.0024 grm.	
Meat, cane sugar, 100 grm. 2 days	150	.00114 "	No crystals in sediment.
Meat, cane sugar	550	.0119 "	Numerous small octahedral crystals.
Meat, 100 grm. sugar	52	Trace.	Numerous octahedral crystals.
Placed on milk Jan. 3.			
Milk, Jan. 5	650	Trace.	No calcium oxalate crystals.
Milk, Jan. 7	500	.0086 grm.	Numerous calcium oxalate crystals.
Milk, Jan. 10	650	.0053 "	
Same, Jan. 12	1750	Specimen lost.	
" " 14	1550	.0056 grm.	A few octahedral crystals.

TABLE VII.

FASTING DOG. STOPPED FEEDING JANUARY 24.

Date.	Vol.	Oxalic acid.	Date.	Vol.	Oxalic acid.
Jan. 25–28	No urine.		Feb. 1	100 cc.	.0043
Jan. 29	60 cc.	.005	Feb. 2	None.	
Jan. 30–31	No urine.		Feb. 3	101 cc.	.0014

In almost every case when a patient was placed upon a diet free from oxalates, the oxalic acid disappeared from the urine, or was present in too small quantities to be of any importance. A marked illustration of the reduction of the oxalic acid excretion by giving a diet free from oxalates is shown in the case of a patient of Dr. S. W. Lambert—a man subject to attacks of renal colic. With a mixed diet, including rhubarb, the twenty-four hours' excretion of oxalic acid was 67.5 mg. When for four days on a diet free from oxalic acid it fell to 4.1 mg.

Cases VIII and IX in Table V were in contrast to all others studied, as when placed on a diet free from oxalates (but rich in carbohydrates)

they continued to excrete an amount of oxalic acid which was above the normal. These were patients in the care of Dr. Hallock of Cromwell, Conn. Case VIII was that of an American woman, unmarried, aged 28 years. She suffered from nervous fears. She had some digestive trouble, at times with headache. Case IX was that of an American woman, aged 54 years, a widow. She had a history of nervous prostration followed by a melancholic condition. She had headache, digestive disturbance and poor sleep. These two cases in contrast with all others examined would definitely indicate that oxalic acid was formed in the body.

To determine whether the ingestion of excessive amounts of carbohydrate food would lead to the production of oxaluria, the following experiment was made:

A dog was placed under observation on Nov. 3, 1899. At that time there was a small amount of oxalic acid present in the urine.[23] The dog was placed on a meat diet, and the urine examined Nov. 18, Nov. 21 and Nov. 25 showed an absence of oxalic acid. On the last named date the dog was placed on large amounts of sugar in addition to meat. The animal took the sugar greedily, at times receiving 250 to 300 grms. in a day. For a month the dog showed no symptoms, but gained rapidly in weight. On Nov. 9 there were noted in the urine a few calcium oxalate crystals, but only a few. From that date until Dec. 27 oxalic acid was absent from the urine or, if present, was in very small amount. In the latter part of December there appeared simultaneously a group of symptoms consisting of loss of appetite, vomiting of frothy mucus, intermittent diarrhœa, the absence of free hydrochloric acid in the gastric juice, the presence of organic acids in the urine, and the precipitation of numerous large calcium oxalate crystals in the urine. On Jan. 1, the dog took almost no sugar, and there were again but few calcium oxalate crystals deposited in the urine. On Jan. 3 very large and very numerous crystals were noted, some appearing in masses of imperfectly formed crystals like microscopic calculi. Whenever the symptoms in this case

[23] To discover the presence of very small quantities of calcium oxalate in the urine, 95% alcohol was added (Salkowski, *Zeitschr. f. physiol. Chem.*, 1886) in the amount of one-third the volume of the urine to be examined. The mixture was then set aside for forty-eight hours, until the calcium oxalate, if present, had crystallized out from the solution. The sediment was then collected from the bottom of the beaker, centrifugalized and examined microscopically. This furnishes a more delicate test for oxalic acid than the complicated quantitative method of analysis.

became severe, the dog would refuse to take its food, when the symptoms (including the oxalic acid excretion) gradually became less marked. The dog was kept on rather large quantities of sugar for six months and throughout that time there was an almost continuous excretion of oxalic acid although the animal received a diet of meat and sugar only.

Two other dogs were treated in a similar way with the same result. These observations indicate that oxalic acid is formed in the animal body.

The question where in the animal organism oxalic acid is formed has been much discussed. Neubauer,[24] Bouchardat[25] and Ellis[26] have claimed that fermentative action in the stomach or intestine may lead to the production of oxalic acid. The most of the writers on this subject have believed that the oxalic acid was formed in the tissues as a result of defective oxidation and that it was probably due to a direct nervous action on the cells.

Oxalic acid, as has been noted above, is produced by many of the higher forms of vegetable life. It has also been noted as a product of lower forms, as of Aspergillus niger. Zopf[27] has described a form of saccharomyces (S. Hansenii, Zopf), found in cotton-seed meal, which in fermentable carbohydrate solutions produces oxalic acid in place of alcohol.

Experiment I.—With Dog I, noted above, the following experiment was made: On Feb. 9 the dog was fed 400 grm. of meat and 200 grm. of glucose with water. The dog vomited one hour afterwards 260 cc. The vomitus consisted of undigested meat suspended in a watery fluid which contained a considerable amount of mucus. There was no free hydrochloric acid. On adding hydrochloric acid and heating, there was a butyric acid odor given off. This vomited matter was found to contain oxalic acid.[28]

[24] Neubauer, op. cit.

[25] Bouchardat, *Annuaire de thérap.*, 1850.

[26] Ellis, *Boston Med. & Surg. Jour.*, 1888, cxviii, p. 64.

[27] Cited from Baumgarten's *Jahresbericht*, 1889, v, p. 452.

[28] The stomach contents were treated with hydrochloric acid and heated over the water-bath 24 hours. They were then filtered, neutralized with ammonia, and rendered slightly acid with acetic acid. A small amount of calcium chloride solution was then added, and 95% alcohol in the amount of one-half the volume of the fluid. The calcium oxalate was precipitated as a finely divided sediment, and only after about two weeks were the characteristic octahedral crystals formed.

Experiment II.—The same dog was given on Feb. 21, 400 grms. of chopped beef and 200 grms. of glucose and the stomach contents removed in 1½ hrs. The result was the same as before. The specimen contained undigested meat and a large amount of stringy mucus. It was acid in reaction, with no free hydrochloric acid. Oxalic acid was present.

Experiment III.—A mixture was prepared in a flask, which contained cane sugar 100 grms., beef ext., 1 grm., water 1000 cc. To this was added 1 cc. of the gastric contents from Experiment I. This was left in the incubator for two days. On examination oxalic acid was found.[29]

Experiment IV.—Dog II. Began feeding sugar, 100 grms. daily on Feb. 23. On March 9, calcium oxalate crystals were found in the urine. On that date there were given 200 grms. cane sugar, which was vomited in a half hour. This specimen was examined for oxalic acid with negative result. On Apr. 5 the dog was given 100 grms. sugar in 250 cc. of water. This was withdrawn from the stomach in 1¼ hrs. The specimen contained meat which was retained in the stomach from an earlier feeding. There was present thick frothy mucus. There was no free hydrochloric acid. Oxalic acid was present.

Experiment V.—On May 11, 1900, there was fed to Dog I 100 grms. of sugar, in 200 cc. of water. This was removed in one hour. The reaction was faintly acid; there was no free hydrochloric acid present; there was found a large amount of thick mucus. Examination for oxalic acid gave a negative result.

Experiment VI.—A portion of the stomach contents obtained in Experiment V was added to a mixture containing sugar 100 grms., beef extract 1 grm., water 500 cc. After fermenting in the incubator for forty-eight hours, oxalic acid was found.

Experiment VII.—A third dog was fed large amounts of sugar daily, beginning Feb. 22. On May 11 the urine was found to contain a

[29] In connection with the fermentation experiments described in this paper, control tests for oxalic acid were made upon uninoculated solutions of Liebig's extract of beef and sugar. These were examined by the same method as the gastric contents (see footnote 28). In fifteen specimens, no oxalic acid was found. In two cases, after diligent search, there was discovered a single small calcium oxalate crystal. The examination of mixtures of chopped beef and sugar for oxalic acid gave negative results. Salkowski (*Berl. klin. Wochenschr.*, 1900, xxxvii, p. 434) records the finding of oxalic acid in beef extract by quantitative analysis. The result of our examinations, however, seemed to prove that, if present, it was in too minute a quantity to affect the validity of the conclusions drawn from the fermentation experiments.

heavy deposit of calcium oxalate crystals. This dog was then fed 100 grms. of sugar in 200 grms. of water and the stomach contents removed in an hour. The reaction was acid. There was no free hydrochloric acid present. There was a moderate amount of mucus. No oxalic acid was found.

Experiment VIII.—A portion of the stomach contents obtained in Experiment VII was added to a mixture containing 100 grms. of sugar, 1 grm. of beef extract and 500 cc. of water. After fermenting in the incubator for two days, oxalic acid was found to be present in the mixture.

Experiment IX.—A mixture was prepared in a flask, consisting of sugar, boiled starch, Huntley and Palmer breakfast biscuits, and water. To this was added 1 cc. of the stomach contents of a patient having persistent oxaluria. There had been no free hydrochloric acid in the stomach contents after a test breakfast. After fermenting in the incubator, oxalic acid was found in this mixture.

Experiment X.—The patient mentioned in Experiment IX was placed on hydrochloric acid given after meals. The calcium oxalate crystals disappeared from the urine almost entirely under this treatment. When the hydrochloric acid had been discontinued for three days, an experiment similar to Experiment IX was made, using a portion of the gastric contents as a ferment. No oxalic acid was formed.

Experiment XI.—To a mixture of 50 grms. of sugar, 0.5 grm. of beef extract and 500 cc. of water there was added 1 cc. of the stomach contents of a patient with marked and persistent oxaluria. After fermenting for two days, oxalic acid was found in the mixture.

The absence of hydrochloric acid in the gastric contents after a test meal, is a noticeable feature in the cases in which the gastric contents were examined. In the case of Dog I, a test was made for free hydrochloric acid, an hour after feeding, on Feb. 9, Feb. 21, Apr. 20 and May 11. Each time the result was negative. In the case of Dog II, the test was made on Mar. 9, Apr. 5 and Apr. 6. Each time there was no free hydrochloric acid found. In the case of Dog III, the stomach contents were examined on March 15 and May 11 and showed an absence of free hydrochloric acid.[30]

[30] In making preliminary experiments to discover an organism producing oxalic acid from solutions of sugar and beef extract (sugar 100 g., beef extract, 1 g., water 500 cc.), it was found that in four specimens, inoculated with baker's yeast, oxalic acid was formed, but in other similar experiments it was not produced. These observations are too few to be of any importance other than to suggest that certain unknown conditions may lead to the formation of oxalic acid through the activity of baker's yeast.

In only three cases was there an examination of the stomach contents of patients having persistent oxaluria. The patient mentioned in Experiment IX had repeated examinations made after test meals, with a constant absence of free hydrochloric acid. The patient mentioned in Experiment XI had long standing oxuluria with local irritative symptoms in the urinary tract. In this case there was no free hydrochloric acid in the gastric juice, and the same result was obtained in a third case with persistent calcium oxalate deposit in the urine.

PHYSIOLOGICAL AND TOXIC ACTION OF OXALIC ACID.

As the presence of oxalate of lime in the urine is found associated with many symptoms, the question naturally arises as to what is the physiological action of oxalic acid and soluble oxalates. Do they produce any of the symptoms of the so-called oxalic-acid diathesis?

Death from oxalic-acid poisoning is ordinarily due to the local corrosive action on the alimentary canal, and large doses may be taken with impunity if they are in well diluted solution. Christison and Coindet[31] say that early in the century oxalic acid was used extensively in making lemonade, and was generally believed to be innocuous. Piotrowski,[32] in experimenting upon himself, took at several different times from 4 to 7 grammes in twenty-four hours with no noteworthy symptoms, and once he took 8 grammes within one hour.

With a view to studying the influence of rather large doses of soluble oxalates, extending over a number of days, the following experiments were made. A healthy man received a diet free from oxalates and was given daily for two weeks from .20 to .50 grm. of ammonium oxalate. This was taken well diluted immediately after meals. The oxalate was given at first alone, then with enough sodium bicarbonate to render the urine but faintly acid, then with dilute hydrochloric acid. The alkali and acid were given to determine the influence of the acidity of the gastric juice upon the absorption of the drug. Table VIII shows the result of the experiment.

[31] Christison and Coindet, *Edinb. Med. & Surg. Jour.*, 1823, xix, pp. 163; 328.
[32] Bucheim, *Arch. d. Heilk.*, 1857, n. F., i, p. 124.

The same experiment was carried out upon another subject with the result shown in Table IX (p. 42).

In the first of these two cases there was no symptom noted excepting polyuria, as indicated in the table. In the second case no symptom whatever followed the ingestion of the drug. In neither case was the excretion of oxalic acid increased above the normal.

TABLE VIII.

HEALTHY MAN. DIET FREE FROM OXALATES. GIVEN AMMONIUM OXALATE.

Date.	Diet.	Sp. gr.	Reaction.	Volume of urine.	Urea.	Uric acid.	Ratio.	Ammon. oxalate taken.	Other medicine.	Oxalic acid in urine.	Sediment after precipitation with alcohol.
Jan. 20	Beef, rice	1016	Acid.	1260	15.4	.359	42.9	None.	None.	.0055	Octahedral crystals abundant.
21	Milk 1200 cc., butter (	1016	"	1545	23.	.487	47.2	"	"	.002	" " small.
22	Huntley & Palmer's biscuits)	1012	"	2035	23.5	.436	53.7	.225 grm.	"	.0059	" " "
23	" " " ..	1014	"	2045	30.	.684	43.8	.35	"	.0065	" " "
24	2 lamb chops, 2 eggs (	1010	"	2410	25.9	.103	25.1	.425	"	.001	" " numerous.
25	As at first.....	1017	Faintly acid.	1565	25.4	.335	75.8	.15	"	.014	Calcium sulphate crystals; small octahedral crystals.
26	" "	1020	"	1280	24.8	.324	76.6	.45	"	.0016	Octahedral crystals numerous.
27	" "	1016	"	2105	28.1	.566	49.7	.45	"	.0051	" " small.
28	" "	1013	"	2680	25.5	.407	62.8	.475	"	.024	" " few.
29	" "	1014	"	2160	31.1	.564	55.	.375	Sod. bicarb. 4.2grm.	.002	" "
30	" "	1013	"	2405	30.8	.515	59.9	.375	4.8	.019	" " very few.
31	" "	1015	"	1975	23.3	.444	52.5	.375	3.6	.036	" " numerous.
Feb 1	" "	1010	"	2400	37.4	.513	72.8	.375	3.6	.0038	" " few.
2	" "	1013	"	2530	34.43	.502	61.3	.350	HCl 3.6cc.	.0057	No octahedral crystals.
3	" "	1010	"	2295	33.9	.437	77.6	.44	5.4cc.	.015	Few " "
4	" "	1012	"	2370	29.8	.157	190.	.30	5.4	.013	Very few " "

Two dogs were given ammonium oxalate, in larger doses, in proportion to body weight, than the men received. The results of these experiments are recorded in Table X (p. 43).

This dog developed no symptoms excepting albuminuria which disappeared whenever the ammonium oxalate was withheld and reappeared whenever it was given. After receiving the dose intraperitoneally, it developed peritonitis and died within a few hours.

Another dog was given ammonium oxalate subcutaneously. On Feb. 28, .5 grm. of ammonium oxalate was given hypodermatically in 100 cc. of water. The dog showed no symptoms for twenty-four hours when there was noted a weakness in the hind extremities. At that time .5 grm. was again injected. There were no symptoms noted excepting increasing loss of power in the extremities until Mar. 4, when the respiration became rapid and panting and the animal died. After the first

TABLE IX.

HEALTHY MAN. GIVEN AMMONIUM OXALATE.

Diet—Bread, butter and meat.

Date.	Amount in 24 hours.	Reaction.	Sp. Gr.	Urea.	Uric acid.	Ratio.	Ammon. oxalate taken.	Other medicine.	Oxalic acid secreted.	Micros. Exam.
Feb. 23	1120 cc.	Acid.	1017	13.48	.6237	21.61	0	0	.00502	No oxalate crystals.
24	945	Faintly acid.	1021	17.32	.6294	27.51	0	0	.00846	Small oxalate crystals.
25	1585	Faintly acid.	1017	25.11	.5016	50.06	.24	0	.0071	Numerous oxalate crystals
26	715	Acid.	1021	14.76	.4706	31.36	.28	0	.0064	" " "
27	815	Acid.	1025	19.18	.3523	54.44	.30	0	.0112	" " "
28	760	Markedly acid.	1022	19.24	.3517	54.91	.30	0	.0007	" " "
Mar. 1	1400	Strongly acid.	1018	14.33	.4199	34.13	.30	Sod. bicarb.	.0067	" " "
2	995	Very faintly acid.	1019	18.58	.5543	33.52	.30	Sod. bicarb.	.0229	Octahedral crystals fewer. [numerous.
3	945	Very faintly acid.	1019	20.03	.5556	36.81	.38	Sod. bicarb.	.0030	Octahedral crystals fairly
4	1740	Very faintly acid.	1016	25.23	.7387	34.22	.42	HCl 5.4 cc.	.0179	Trace of sulphates left. Numerous cal. oxal. crystals and cal. sulphate.
5	1165	Faintly acid.	1018	18.62	.3132	59.48	.42	HCl 5.4 cc.	.0074	Calcium oxalate crystals not very numerous.
6	2100	Faintly acid.	1009	15.45	.9076	17.02	.48	HCl 5.4 cc.	.0087	Very few calcium oxalate crystals.

injection of ammonium oxalate there was no urine voided for forty-eight hours. Then 220 cc. were passed. This contained no albumin, no sugar nor other reducing agent. .1466 grm. of oxalic acid was recovered. The next day 250 cc. of urine were passed, containing a trace of albumin, no sugar or other reducing agent. There was found .078 grm. of oxalic acid. On the next day 690 cc. were voided containing .057 grm. of oxalic acid. There was thus given in all, subcutaneously, 1 grm. of ammonium oxalate, which represents .726 grm. oxalic acid. Of this there was recovered in the urine .281 grm. or 37%.

The autopsy on this dog was made one-half hour after death. The blood was still fluid throughout the body, but it slowly coagulated upon exposure to the air. The heart was in diastole, filled with liquid blood,

with a single small clot in the left ventricle. The lungs showed areas of congestion. The most interesting lesion was in the kidney. Here the section showed numerous crystals blocking up the uriniferous tubules. Most of them were of irregularly shaped masses. Some showed characteristic dumbbell and ovoid shapes. These crystals were of a very light yellow color, were unstained by eosin or hæmatoxylin, were insoluble in acetic acid and ammonia, but dissolved in dilute hydrochloric acid. There were no changes in the glomeruli. The cells lining the tubules were in places swollen and granular, and in places were torn away by the passing of a calculus.

TABLE X.

ADMINISTRATION OF AMMONIUM OXALATE TO DOG.

Date.	Amount of urine.	Albumin.	Ammon. oxalate taken.	Oxalic acid taken in form of ammon. oxalate.	Amount of oxalic acid excreted.
Feb. 4....		None.	.50 grm.	.36	
Feb. 5....		"	.50 grm.	.36	
Feb. 6....	144 cc.	"	0	0	.0285
Feb. 7....		"	.75	.54	
" 8....	315 cc.	"	1.00	.72	Specimen lost.
" 9....		"	1.00	.72	
" 10....	175 cc.	"	1.00	.72	.0242
" 11....		"	1.00	.72	
" 12....		Trace.	1.00	.72	
" 13....	565 cc.		1.00	.72	.0707
" 14....		None.	0	0	
" 15....	450 cc.		0	0	.0464
" 16....			.4	.29	
" 17....	90 cc.	Trace.	.4	.29	.0035
" 18....			0	0	
" 19....			0	0	
" 20....	980 cc.	None.	0	0	.0034
" 21....			0	0	
" 22....			0	0	
" 23....			0	0	
" 24....	110 cc.	Large amount.	1 grm. intraperitoneally.	.72	
" 25...					

The study of acute oxalic acid poisoning does not come within the scope of this paper. It may, however, be noted, in connection with the above experiments, that the symptoms of the acute poisoning have been carefully studied by Christison and Coindet,[33] R. Koch,[34]

[33] Op. cit.

[34] R. Koch, *Arch. f. exp. Path. u. Pharm.*, 1881, xiv, p. 153.

Kobert and Küssner,[35] and Krohl,[36] and the pathology has been studied by A. Fraenkel[37] and by Ebstein and Nicolaier.[38] The most common symptoms are those of the nervous system: either irritative phenomena, as muscular twitchings and tonic or clonic convulsions; or paralytic phenomena—paresis or paralysis, both motor or sensory, with abolition of reflexes, and excessive lowering of temperature before death. The pulse, respiration and blood-pressure are not influenced except in fatal cases, and there are no digestive symptoms unless the drug is given by mouth. Several observers have found in the urine a strong reducing agent, acting upon copper sulphate and bismuth subnitrate, which does not give the test for sugar by the spectroscope or polariscope. The urine may contain albumin or casts or red corpuscles or any of the forms of calcium oxalate crystals.

On the Oxidation of Oxalic Acid in the Organism.—The slight danger in taking large doses of soluble oxalates seems to be due in part to the precipitation of the oxalic acid as calcium oxalate in the alimentary canal, and its remaining unabsorbed in the fæces; and in part to the oxidation in the organism of that which is absorbed. Gaglio,[39] experimenting with a cock, recovered from the fæces all the oxalic acid that was taken; but Guinti,[40] while verifying Gaglio's experiment in the case of the cock, found that in dogs and man, a portion of that absorbed was oxidized.[41]

In the experiments described above it will be noted that in the cases of the men receiving ammonium oxalate, in amounts varying from .2 grm. to .48, and extending over a period of eleven days in

[35] Kobert and Küssner, Virchow's *Archiv*, 1879, lxxviii, p. 209, and 1880, lxxxi, p. 383.

[36] Krohl, *Arb. a. d. pharmakol. Inst. z. Dorpat*, 1891, vii, p. 130.

[37] A. Fraenkel, *Zeitschr. f. klin. Med.* 1881, ii, p. 664.

[38] Ebstein and Nicolaier, Virchow's *Archiv*, 1897, cxlviii, p. 366.

[39] Gaglio, *Arch. f. exp. Path. u. Pharm.*, 1887, xxii, p. 235.

[40] Guinti, *Ann. di chim. e di farm.*, Milan, 1897.

[41] Thus in giving a man .31 grm. he recovered in the fæces .038 grm., leaving .272 absorbed. Of this there was excreted in the urine during the first two days .062 grm. or 11%. From this he concludes that the rest was oxidized in the system. In a similar experiment on a dog he recovered 11% of that absorbed. But in giving the drug hypodermatically to a dog, he recovered at one time 43.6%, and again 51% of that injected.

one case, and two weeks in the other, only traces of oxalic acid appeared in the urine, while in the case of the dog receiving the drug subcutaneously 37 per cent was recovered. As no study was made of the fæces in these experiments it is impossible to form any opinion as to whether any oxalic acid was oxidized in the body.

CONCLUSIONS.

1. As varying amounts of calcium oxalate may be held in solution in the urine, conclusions based upon the presence or number of calcium oxalate crystals found therein are of no real value as an indication of the quantity of oxalic acid present.

2. Unless the utmost care is exercised, the results obtained by quantitative estimation of oxalic acid are subject to large percentages of error. This is especially true in the use of Neubauer's or Shultzen's methods, in which the calcium oxalate is precipitated in an alkaline solution.

3. An ordinary mixed diet regularly contains traces of oxalic acid or its salts.

4. A portion of the oxalic acid ingested with the food may be absorbed and reappear unchanged in the urine.

5. The normal daily excretion of oxalic acid in the urine fluctuates with the amount taken in the food, and varies from a few milligrammes to two or three centigrammes, being usually below ten milligrammes.

6. In health, no oxalic acid, or only a trace, is formed in the body, but that present in the urine has been ingested with the food.

7. In certain clinical disturbances which in some of the cases studied above were associated with absence of free hydrochloric acid from the gastric juice, oxalic acid is formed in the organism.

8. This formation in the organism is connected with fermentative activity in the alimentary canal.

(*a*) The prolonged feeding of dogs with excessive quantities of glucose, together with meat, leads eventually to a state of oxaluria.

(*b*) This experimental oxaluria is associated with a mucous gastritis, and with absence of free hydrochloric acid in the gastric contents.

(*c*) The oxaluria and the accompanying gastritis are referable to fermentation induced by the excessive feeding with sugar.

(*d*) The experimental gastritis from fermentation is associated with the formation of oxalic acid in the gastric contents.

9. The symptoms attributed to an oxalic acid diathesis, with the exception of those due to local irritation in the genitourinary tract, do not appear to be due to the presence in the system of soluble oxalates, but are more likely to depend on other products of fermentation and putrefaction.

I wish to express my gratitude to Dr. Herter, at whose suggestion and under whose guidance this study has been made, and to Dr. A. J. Wakeman for advice and aid in carrying on the work.

SERUM-GLOBULIN AND DIPHTHERIC ANTITOXIN.—A COMPARATIVE STUDY OF THE AMOUNT OF GLOBULIN IN NORMAL AND ANTITOXIC SERA, AND THE RELATION OF THE GLOBULINS TO THE ANTITOXIC BODIES.[1]

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The varying statements concerning the amount of globulin in serum from immunized and non-immunized horses, and concerning the relation of the antitoxic principle to this constituent and to other constituents of serum, leave no doubt as to the confusion existing in regard to these questions, and indicate a promising field of research.

The propositions upon which our work was primarily based were the following: To analyze normal sera and antitoxic sera of different values for the determination and comparison of the amounts of globulin and albumin, and of the antitoxic values of these globulins and albumins, and also of the various filtrates accompanying their preparation.

In the analysis of blood-serum various methods have been devised and recommended for obtaining the different ingredients of the serum in a pure state. Of these methods, those recommended for the precipitation of the globulins are of chief interest to us and will be briefly considered. The principal methods are the following: (*a*)

[1] Received for publication February, 1900. The experiments recorded in this paper were begun in May, 1897, and have extended over the intervening years. They have been performed at the Research Laboratory of the Department of Health of New York City, where exceptional opportunities for such investigations exist, owing to the large number of horses constantly undergoing immunization for the production of diphtheric antitoxin.

precipitation of the globulins by super-saturation of the serum with magnesium sulphate or semi-saturation with ammonium sulphate; (*b*) precipitation by means of CO_2 gas, by passing it through solutions containing the serum or globulins; and, (*c*) finally, precipitation by dialysis, this method depending for its success upon the removal of the natural or added salts, the globulins being looked upon as insoluble in pure water and hence precipitated by removal of the salts.

Dieudonné[2] has shown that marked differences exist in the action of the globulins prepared by different methods, when tested for their antitoxic power. Globulins obtained by precipitation with CO_2, according to him, display the least antitoxic value; those obtained by precipitation with magnesium sulphate the greatest; while globulins, the product of dialysis, hold an intermediate place in the antitoxic scale.

In his experiments, Dieudonné used a normal horse's serum which, he says, showed marked antitoxic properties. One cubic centimetre of this serum mixed with twice the fatal dose of diphtheric toxin, in vitro, protected the animal even from marked local symptoms. Globulin prepared from this serum by CO_2 gas was found to have practically no antitoxic value. This result differed so widely from the results claimed by Smirnow,[3] that another experiment was undertaken in which the globulins were precipitated by the addition to the serum of an excess of magnesium sulphate. This was the method that Smirnow had employed, and Dieudonné found "the preparation obtained by the magnesium sulphate acted very differently from that prepared by carbonic acid; it showed toxin-neutralizing properties like those obtained by Smirnow in his experiments with globulin." Further experiments brought to light the fact that globulins won by means of dialysis were markedly less active than the precipitate obtained by magnesium sulphate, but that the filtrate had a noticeable antitoxic value.

Dieudonné concludes from his experiments "that the antitoxic property does not belong to the globulin, but to some unknown body contained in the serum which in the preparation of the globulin is carried down mechanically in the precipitate, or becomes closely bound up with it; and that by the rapid precipitation with magnesium sulphate this

[2] *Arb. a. d. k. Gesundheitsamte*, 1897, xiii, p. 297.

[3] *Arch. d. sciences biol.*, St. Pétersb., 1895, iv, p. 328.

active substance is much more easily seized, than in the slower separating out of the globulins by carbonic acid or by dialysis, in which only a small portion is taken with the precipitate." This active body contained in serum must therefore, he concludes, be least precipitable by carbonic acid, somewhat more precipitable by dialysis, and most precipitable by magnesium sulphate; and hence the action of the various filtrates obtained in the different methods of globulin preparation is also different. After precipitation by magnesium sulphate these fluids are poorest in antitoxic bodies; after carbonic acid preparation, richest. This becomes especially apparent if the following experiments of Dieudonné are considered.

The precipitate previously obtained by magnesium sulphate was redissolved and subjected to dialysis in flowing and distilled water. The precipitate thus obtained by dialysis was redissolved in 2% salt solution. The original magnesium sulphate globulin precipitate had a high protective value, while even 2 cc. of the fluid remaining after the abstraction of the globulin was totally inactive. These proportions were very different after dialysis. The globulin precipitated by dialysis was only weakly active, while of the fluid freed from the globulin, 0.5 ccm. still displayed marked neutralizing powers. These differences are more apparent still in the second experiment, *i. e.*, the purification of the magnesium sulphate globulin by means of carbonic acid gas. One gramme of the dried magnesium sulphate globulin was dissolved in 50 cc. of water. In passing CO_2 gas directly into this solution not a trace of precipitate was to be noted. The solution was therefore subjected to dialysis, first in running water, then in distilled water, until no trace of sulphuric acid could be determined. In the parchment tube an abundant precipitate appeared, which was redissolved in 2% sodium chloride solution. Into this solution carbonic acid gas was run for two hours and a fine flocculent precipitate obtained. In testing the antitoxic value of this precipitate it was found to have lost its value and that the antitoxin was for the most part in the filtrate instead of with the globulin as in the original magnesium sulphate precipitation.

These experiments have seemed worth detailing in this connection since an explanation of the results obtained has been advanced by Seng[4] in his recent paper. Seng mentions that Brieger's[5] observation that the antitoxins from zinc chloride precipitation go over into the filtrate from reprecipitation with carbon dioxide, while those from zinc sulphate

[4] *Zeitschr. f. Hygiene*, 1899, xxxi, p. 513.

[5] Ibid., 1896, xxi, p. 267.

precipitation remain with the precipitate when reprecipitation by carbon dioxide is practiced, demonstrates that the chemical nature of the resulting precipitate or of the precipitating method decides whether the antitoxin falls with the precipitate, but shows that it is in no wise carried down by the precipitate.

He cites experiments in support of this view and against Dieudonné's conclusion that the antitoxins are mechanically carried down with the precipitates, and says: "That a further weighty support to this view was brought by the researches of Dr. Sternberg," who, finding that "it was inconvenient to work with such richly albuminous antitoxin solutions as those composing the native diphtheric serum," was able to show "that the albumin could be removed by other than the Brieger method from the antitoxin, and that by the addition of $\frac{1}{3}$ vol. of a 5% (concentrated) solution of potash alum to the serum, a great part of the albuminous bodies would be precipitated as an abundant, voluminous, precipitate, the antitoxins, however, remaining in solution."

Here, then, the antitoxins remained in solution in spite of a voluminous precipitate, and by further experiments it was shown that no matter whether the albumins were previously removed or whether the globulins were precipitated by magnesium sulphate or ammonium sulphate, nevertheless, the entire antitoxin always remained with the globulins.

The vital point in Seng's experiments was reached in removing the excess of the precipitating salts from the globulins. The removal of the salts was accomplished by dialysis, and it was to be expected, according to previous conceptions, that, by a dialysis which was carried on until all traces of chlorine, ammonia, and sulphuric acid reaction had disappeared, all globulins would be precipitated in the salt-free solution, but it appeared that only a very small part, 1:23 to 1:11, of all the globulin separated itself out as insoluble, the majority of the globulin and with it the entire antitoxin remaining in solution. It was determined by experiment that the antitoxins clung to these "soluble globulins."

This may be the explanation of the discrepancies noted when globulins obtained by different methods are tested for their antitoxic value, the antitoxin remaining apparently with the "soluble globulins" only. Both the soluble and the insoluble globulins are precipitated by magnesium sulphate, but only the insoluble by dialysis.

Early in our own work it became apparent that the magnesium sulphate method was the most trustworthy known to us for obtaining

globulins and the antitoxic principles of the serum. Throughout the paper, therefore, the results of precipitation by magnesium sulphate will alone be given.

In obtaining the globulin content of the various sera, both normal and antitoxic, the following method has been followed:

Separation of the globulins from blood-serum.—10 cc. or 20 cc. of the serum was diluted to 50 cc. or 60 cc. with a saturated solution of magnesium sulphate. Crystals of magnesium sulphate were then added and the mixture stirred until completely saturated. When precipitation was complete, the precipitate was filtered out and redissolved in water together with the crystals of magnesium sulphate still remaining in the original beaker glass, the quantity of solution thus resulting amounting generally to 350 cc. to 400 cc. From this solution the globulin was again precipitated with magnesium sulphate and filtered out, the beaker being washed with a saturated solution of magnesium sulphate, which fluid was then poured onto the filter. The globulin thus obtained was washed on the filter paper with a saturated solution of magnesium sulphate, and finally redissolved in distilled water.

These precipitates were then tested upon guinea-pigs in order to determine their antitoxic values and to compare these with the values of the sera from which they were derived.

All tests of serum for antitoxic value were made by mixing the toxin and serum and then injecting the mixture into the test animal.

Determination of the antitoxic value of the globulins.—In making the tests for antitoxin in globulin solutions, exactly the same method was followed as that employed in testing serum itself. The globulin from the 10 cc. or 20 cc. of the serum was dissolved in a known quantity of sterile distilled water. Of this solution a quantity corresponding in globulin content to 1 cc. of the serum, from which it was derived, was diluted to the required strength and mixed as usual with the 10-times fatal dose of toxin.

Table I shows the results of the tests with globulins. Some tests of albumins from the sera are recorded in Table II.

Toxin control animals for all these tests died in the usual time, 4 to 7 days.

From Tables I and II it can be readily seen that the tests of the globulins obtained by magnesium sulphate precipitation show that practically the total antitoxin content of the various sera is associated

with the globulins, these globulins showing an antitoxic value equal to the strength of the antitoxin contained in the serum from which they were derived.

TABLE I.

DETERMINATIONS OF ANTITOXIC VALUE OF GLOBULINS FROM ANTITOXIC SERA.

Date.	No. of Horse.	Units of Antitoxin in 1 ccm. of serum.	Units of Antitoxin tested for in glob. from 1 ccm. of serum.	Weight of test Guinea Pig.	Result of Test.
26–III–'97 ..	83	500	500	278	Animal not affected.
30–XII–'97..	105	500	500	270	" " "
1–XII–'97..	112	550	550	230	" " "
1 " '97..	112	550	550	228	" " "
1 " '97..	112[6]	550	550	260	" " "
1 " '97..	112[7]	550	550	237	" " "
3–VI–'97 ..	128	500	500	218	" " "

TABLE II.

DETERMINATIONS OF ANTITOXIC VALUE OF ALBUMINS.

Date.	Horse.	Units of Antitoxin in 1 ccm. of serum.	Units of Antitoxin tested for in Albumin from 1 ccm. of serum.	Weight of test Guinea Pig.	Result of Test.
26–III–'97 ..	83	500	5	222	Animal died in 5½ days.
3–VI–'97 ..	128	500	5	272	Animal died in 1½ days.

To determine the action of magnesium sulphate by itself upon the toxins, and thus rule out any error that might arise from such action, if present, the following experiment was undertaken:

A solution of magnesium sulphate calculated to be the same in strength as would be found with solutions of the globulins was mixed with toxin as follows and injected into guinea-pigs:

TABLE III.

TO DETERMINE ACTION OF MAGNESIUM SULPHATE ON TOXIN.

Weight of test Guinea Pig.	ccm. of $MgSO_4$ sol.	Fatal doses of toxin.	Result of test.
225 gm.	2 ccm.	2	Died in 3 days.
" "	2 "	4	" " 2½ days.
" "	2 "	6	" " 2½ days.

[6] Same globulin solution as 112, but kept exposed to light at room temperature in stoppered blue-glass bottle for 2½ months.

[7] Same globulin solution as 112, but kept in bottle in ice-chest three months before testing.

As is evident from Table III, no neutralizing action upon the toxin was determined, the test animals dying in about the usual time; we may also conclude from these experiments that the $Mg\ SO_4$ had no stimulating effect upon the animal.

To determine, on the other hand, the evil effects of injecting $Mg\ SO_4$ into guinea-pigs the tests recorded in Table IV were made:

TABLE IV.

To Determine Injurious Effects of Injection of Magnesium Sulphate.

Weight of test Guinea Pig.	ccm. of sat $Mg\ SO_4$ sol.	Result of test.
300 gm.	0.5 ccm.	Animal not affected.
335 "	0.25 ccm.	" " "
307 "	0.1 ccm.	" " "
250 "	1 ccm.	" died in 10 minutes.

As the globulin from 1 cc. of an antitoxic serum of 500 units strength, when tested for this strength, is diluted 5000 times, it is plain from the above results that the amount of magnesium sulphate injected into an animal with the globulin solution must be too small in quantity to have any appreciable effect on the animal.

Having, by the foregoing experiments, demonstrated to our satisfaction that the magnesium sulphate precipitates contained practically all the antitoxic bodies—be these globulins or bodies precipitated with them in the presence of magnesium sulphate—we were then in a position to carry on the major part of our work, *i. e.* the comparison of the weights of these precipitates as obtained from the serum of non-immunized and of immunized horses at various stages of resistance. These determinations were made in the manner detailed below, the greatest care being taken to avoid error, several determinations often being made from the same serum.

Coagulation and weighing of the total coagulable albuminous bodies (proteids); globulins, and albumins.—The globulin and albumin were coagulated together from the horse serum in the following manner: 10 cc. or 20 cc. of the serum, as the case might be, were diluted to 350 cc. or 400 cc. with water, and the temperature gently raised over the Bunsen burner until coagulation began. The temperature was then quickly raised to boiling point, the solution being constantly stirred. When

the solution was boiling well, dilute acetic acid was added drop by drop until a faint acidity was reached. After a few moments more of boiling, the coagulation was complete and the flocculent mass quickly settled to the bottom of the beaker glass. The coagulated proteids were filtered onto a weighed filter paper, washed with boiling water, and, finally, slowly dried in the air bath to constant weight.

The globulin obtained by precipitation with $Mg\ SO_4$ was coagulated and filtered from its watery solution in precisely the same manner. The difference between the weight of the globulin and the total albuminous (proteid) precipitate was considered as the weight of the albumin in the serum taken. This method is open to some objections, but with careful technique very accurate results for comparison may be obtained.

Table V gives the results of these experiments. The determinations are given in grammes.

A consideration of the figures of Table V convinces us that although there is no absolute conformity in the amount of the precipitates from sera of the same antitoxic value, still even in the comparison of these sera from different horses it is apparent that the lower the antitoxic value of the serum the lower is the globulin content, *i. e.* if normal serum, according to our determination for 20 ccm., gives 0.8 grm. of precipitate, then 200-units serum will average much higher, 300 higher still, and so on, if a long series is taken. But there were such marked exceptions to this rule that it was evident that a high antitoxic value is possible in a serum of comparatively low globulin content, as low often as a serum of practically no antitoxic value. The absolute amount was then no index of antitoxic value. This dispelled our hope of determining before undertaking immunization the value of a horse for the production of antitoxin by globulin determinations.

It early became evident, except for the interest of general statistics and comparison, that determinations based upon the sera of different horses were of little value, and that the only safe data upon which to found conclusions would be those obtained from the serum of the same animal, from the normal state on to a high degree of immunity. In several cases it has been possible to make such determinations.

From Table VI it is obvious that, no matter what the initial globulin content, this amount was always increased as the immunization

TABLE V.

DETERMINATION OF TOTAL ALBUMINOUS BODIES (PROTEIDS)—GLOBULINS AND ALBUMINS—FROM SERA OF DIFFERENT ANTITOXIC VALUES.

Date.	Horse.	ccm. of serum used.	Antitoxic units per ccm.	Weight of globulin.	Estimated wt. of albumin.	Weight of albuminous bodies (glob. and alb.) precipitated by heat.
16-XI-'97.....	7	20	300	1.4077	.4928	1.9005
18-XII-'97....	"	"	400	1.5548	.5194	2.0742
11-V-'97	83	"	200	.9834		
29-VI-'97.....	"	"	400	1.3344	.2354	1.5698
?	"	"	500	1.4683	.3959	1.9642
6-XII-'97	"	"	850	1.5298	.4695	1.9993
?	89	"	Normal.	.5710	.4707	1.0417
21-V-'97	91	"	200	.7358	.3263	1.0621
13-IV-'97.....	"	"	300	1.1720	.3928	1.5648
?	95	"	Normal.	.7265	.6465	1.3730
8-XII-'97	96	"	300	1.3540	.4123	1.7663
29-XI-'97	"	"	400	1.5949	.5143	2.0806
27-IX-'97	98	"	Normal.	.6171	.8057	1.4228
?	99	"	"	.7593		
?	100	"	"	.8150	.5610	1.3760
?	101	"	"	.7602		
?	101	"	200	1.4193		
?	103	"	Normal.	.8685	.6535	1.5220
21-IV-'98.....	108	"	"	.6743	.6871	1.3614
	"	"	200	.8436		
?	109	"	Normal.	.5818	.8376	1.4194
10-XII-'98.....	110	"	"	.7559	.6834	1.4393
	110	"	300	1.2101	.5773	1.7874
27-XI-'98.....	111	"	Normal.	.6377	.8221	1.4598
	"	"	500	1.5447	.2455	1.8902
	112	"	450	.7569	.7281	1.4850
	"	"	500	1.0591	.5918	1.6509
	116	"	Normal.	.7977		
	117	"	"	.9943	.6826	1.6819
	118	"	"	.7773	.7373	1.5146
	119	"	"	.7673	.6532	1.5205
	"	"	150	.8056	.5908	1.4964
	121	"	Normal.	1.1429	.5944	1.7373

proceeded, although no fixed amount of increase seemed to represent a given number of units of antitoxic value. The same thing is apparent in Table VII, where lists are given of determinations from horses in whose case it was impossible to obtain data concerning the normal serum.

TABLE VI.

GLOBULIN DETERMINATIONS FROM NORMAL AND ANTITOXIC SERA.[8]

		Units of Antitoxic Strength.				
	Normal.	150	200	300	400	500
Horse 89.....	.5710					
" 95.....	.7265					
" 98.....	.6171					
" 99.....	.7593					
" 100.....	.8150					
" 101.....	.7602		1.4193			
" 103.....	.8685					
" 108.....	.6743		.8436			
" 109.....	.5818					
" 110.....	.7559			1.2101		
" 111.....	.6377					1.5447
" 116.....	.7977					
" 118.....	.7773					
" 119.....	.7673	.8056				
" 121.....	1.1429					

We again find demonstrated the excess of the magnesium sulphate precipitates from the sera of horses which have reached high degrees of immunization over the sera of the same animals at a less advanced stage.

TABLE VII.

GLOBULIN DETERMINATIONS FROM ANTITOXIC SERA.[9]

	200	300	400	450	500	550	600	650	700	750	800	850
Horse 7		1.4077	1.5548									
" 83			1.3344		1.4683							1.5297
" 91	.7358	1.1720										
" 96		1.3540	1.5949									
" 112				.7569	1.0591							

In Table VIII are given some globulin and albumin determinations made from 10 cc. amounts of various sera. The globulins were pre-

[8] Determinations made from 20 cc. of serum, and results given in grammes.

[9] Determinations made from 20 cc. of serum, and results given in grammes.

cipitated with Mg SO_4 in the regular manner; and the albumins were then precipitated from the filtrate by heat. The precipitates were collected, however, on Gooch crucibles instead of on filter-papers, and the weighings thus made with probably less error than in the methods used by us earlier in making these determinations. Nevertheless, remembering that we are dealing with 10 cc. instead of 20 cc. amounts of serum, we find the results practically unaltered. It is interesting to note the decrease shown by the albumins as the globulins increase. Whether this is a coincidence, or a regular gain of the one at the expense of the other, it would be difficult to determine from our few tests. If it is a constant occurrence, it may be that a transmutation takes place, or simply that the process which regulates the albumin content is depressed during the period of exaltation of globulin production.

TABLE VIII.

GLOBULINS.

Horse.	Normal.	400	500	600	650	1200
133	.3727	.7782				
135	.3864	....	.8987			
136	.3566	....	....	.7389		
137	.3235	.4743	....	.5116	.5934	.8987

N. B.—Horse No. 137 went from normal to 1200 units at first bleeding; subsequent bleedings with no intervening toxin inoculation showed constant decrease in the number of units, likewise in globulin.

ALBUMINS.

133	.4146	.2102				
135	.3680	....	.2341			
136	.3618	....	....	.2715		
137	.3127	.3133	....	.2938	.2727	.2341

TABLE IX.

GLOBULIN DETERMINATIONS FROM SERUM OF HORSE 83.

	200	300	400	500	850
Horse 83.....	.9834				
" ".....	.9666	....	This serum dropped from 700 units.		
" ".....	....	1.0218	" " " " 500 "		
" ".....	....	....	1.3344		
" ".....	....	....	1.1478	This serum dropped from 600 units.	
" ".....	....	....	....	1.4683	
" ".....	....	....	....	....	1.5297
Average.	.9750	1.0218	1.2411	1.4683	1.5297

The comparison given in Table IX is exceedingly interesting. The table shows the determinations from one of the horses that has long been a source of high grade antitoxin. Figures are given for the magnesium sulphate precipitates from 200 unit serum to that containing 850 units, with the difference of 0.5 grm. between the determination of 200 unit serum and the 850 unit specimen.

The most interesting point in this connection is that some of these specimens when first drawn and tested gave a high number of units, and that, after having been kept, they dropped very considerably in their antitoxic value and globulin content, this being due possibly to some obscure chemical change or more probably to contamination with microörganisms. Comparing two 200-unit specimens, one of which had dropped from 700 units, and the other having been freshly drawn, we find their globulin content practically the same. A 400-unit specimen, which had dropped from 600 units, showed a like decrease in the amount of globulin; likewise a 500-unit serum, which had fallen to 300 units, gave a precipitate in weight between that of the 200 and 400-unit specimens.

Although we do not feel justified in positively concluding from the foregoing facts that the antitoxin, or antitoxic bodies as we prefer to call them, are globulins, yet we cannot help feeling that such facts point very strongly to their being globulins, or at least bodies which have in all points, in the light of our present knowledge, the same reactions as the globulins. It seems a remarkable coincidence that the antitoxic bodies and the globulins should have been destroyed in such exact proportion in those sera which had lost in antitoxic value, if they were not the same bodies.

Undoubtedly included under the general term "serum globulin" there are several or many bodies of slightly varying chemical characters that eventually we may be able to distinguish one from the other, and determine their absolute amounts. Some of these globulins, it seems not unlikely, may, at times, be "cell globulins" in contradistinction to the true "serum globulins," and be found in the serum after some unusual destruction of the formed elements of the blood.

The variations in the initial amounts of globulin present in the serum of different animals may be due to normal variations in the physiological processes, but many of them are possibly to be explained as pathological conditions, and among these may be increases due to various past infections through which the animal has successfully passed, or to minor infections, less obvious, but existing before and at the time of the taking of the specimen for analysis. All such complicating conditions should be taken into consideration, and an attempt made to reduce results to uniformity by excluding such complications or allowing for their existence when making comparisons.

In considering initial amounts of globulin the antitoxic value of so-called "normal serum" in each case should be carefully determined, as a partial explanation of variations in initial amounts of globulin may be found in the variations in the protective value of these sera. In our work this has not been done in all cases, but Table X gives the results of some tests undertaken to determine the neutralizing power of serum from normal horses, and Table XI the results in determining the neutralizing power of the globulin from this normal serum. It will be seen that many among these display a marked neutralizing action when tested against one or more fatal doses of diphtheric toxin. The accepted unit of antitoxin—the amount of antitoxin which will protect the test animal against 100 minimal fatal doses of the diphtheric toxin—represents in reality a fair neutralizing action, for 1 cc. of a serum possessing only a strength of one unit has the power of rendering harmless 100 minimal fatal doses of toxin. Remembering this in considering these tables, it will be seen that, although these sera show only very exceptionally as high a power as one antitoxic unit, yet some of them in amounts of one ccm. protect the test animal (guinea-pig, 250 grm.) against many minimal fatal doses of the toxin—in the case of Horse 137, against 300 minimal fatal doses. Reference to Table XI will also show that the globulins from these sera act in a like manner against the toxins of diphtheria. These sera, however, in many instances do not contain more globulin than others of less neutralizing power, hence the variations in diphtheric antitoxin or neutralizing substances are again demon-

strated to be only one of the factors causing increase in the globulin precipitates, and can but partially explain the variations in these precipitates.

TABLE X.

DETERMINATIONS OF THE NEUTRALIZING POWER OF NORMAL SERA.

Horse.	Weight of Guinea Pig.	Serum + Toxin.				No. of fatal doses protected against.	Result.
126	225	10	cc. +	1.5	f.d.t.[10]		Loss in wt.; no induration. Recovered.
"	315	5	" +	"	"		Died in 3 1/2 days.
127	280	5	" +	"	"		No loss in wt.; no induration.
"	335	3	" +	"	"	0.5	Loss in wt.; induration. Recovered.
"	320	1	" +	"	"		Died in 3 1/2 days.
135	365	1/2	" +	"	"		No loss in wt.; no induration.
"	270	1/10	" +	"	"	15	Induration; loss in weight.
"	248	1/20	" +	"	"		Died in 4 1/2 days.
"	243	1/2	" +	5	f.d.t.		?
136	374	"	" +	1.5	"		Much induration.
136	240	1/4	" +	"	"		Much induration; loss in weight.
137	255	1/20	" +	1.5	"	30	Gain in weight; no induration.
"	260	1/5	" +	5	f.d.t.	25	No induration; no loss in weight.
137	270	1/10	" +	10	"	100	" " "
"	225	1/100	" +	1.5	"	150	" " "
"	250	1/30	" +	10	"	300	" " "
"	254	1/35	" +	10	"		Died in 3 1/2 days.
139	355	3	" +	1.5	"		Much induration; slight loss in wt.
"	325	1	" +	"	"		Died in 48 hours.
140	270	10	" +	"	"	0.15	Induration; slight loss in weight.
"	340	5	" +	"	"		Died in 3 1/2 days.
141	270	1/4	" +	"	"	6	No induration; gain in weight.
"	275	1/10	" +	"	"		Loss in wt.; induration.
"	255	1/20	" +	"	"		Died in 3 1/2 days.
142	313	10	" +	"	"		No induration.
"	365	5	" +	"	"	0.3	Induration; no loss in weight.
"	325	3	" +	"	"	0.5	Loss in weight; induration.
"	325	1	" +	"	"		Died in 3 1/2 days.

All controls of 1 1/2 f.d.t. died in 2 1/2 to 3 1/2 days. Toxic dose corrected for weight in all tests.

It is then not at all surprising that the amount of so-called normal globulin should be a very varying one if we keep in mind all the various factors which must certainly influence its increase and decrease; nor is it curious that no definite amount of increase can be determined for a given increase in antitoxic value, since many of

[10] f. d. t.=minimal fatal dose of toxin.

these same causes are constantly at work masking results. More refined methods of analysis and separation may bring out differences between the substances increased and show us that there is a constant factor, but at present we cannot say more than that the globulins and antitoxin increase and decrease under the same conditions, and respond in the same manner to all tests.

Although the determination of the protective value of sera from normal horses was undertaken by us principally to establish some relation between these values and the amount of globulin precipitate obtained from the sera, still we hoped also that such determinations might in themselves serve as indications of the value of horses for the production of diphtheric antitoxin.

TABLE XI.

DETERMINATIONS OF THE NEUTRALIZING POWER OF THE GLOBULINS FROM NORMAL SERA.

Horse.	Weight of Guinea Pig.	Globulin sol.[11] and Toxin.	No. of fatal doses protected against.	Result.
135	270	1/2 cc. + 1.5 f.d.t.	3	Gain in weight; no induration.
"	245	1/2 " + 5 "		Died in 7 1/2 days.
137	244	1/5 " + 5 "	25	No induration; no loss in weight.
"	260	1/30 " + 10 "	300	" " " " "

An analysis of the results obtained is so dependent upon one's conception of the relation of the normal protective or neutralizing substances to the true antitoxin, and also upon one's conception of the origin of antitoxins, that some reference to these questions is necessary, although it must be remembered that any discussion of these subjects is at present necessarily largely hypothetical.

It may be generally true that normal horses, the sera of which possess marked neutralizing powers against the diphtheric toxin, are more promising subjects for immunization than are those from whose sera such power is practically absent or only poorly developed; yet this is not the constant experience of ourselves or of others,[12] and

[11] Globulin solution contains the same amount of globulin in 1 cc. as was contained in 1 cc. of the serum from which it was derived.

[12] See Bolton, *Journal of Experimental Medicine*, 1896, I, p. 543.

directly opposed to it has been our experience especially with one of our horses. In this case the normal serum showed quite a marked neutralizing action, while, even after long treatment with toxins, only a very weak antitoxic serum was obtained, and the animal, as a source of antitoxin, was abandoned. Such occurrences make us feel that it is possible for the animal body to take care of a poison by other methods than the increased production of anti-substances, for instance by an unusual power of excretion of the poison, or by rendering it inert by destruction rather than by antagonistic action or neutralization. It seems well at least not to allow our conceptions to become too narrow when dealing with these special problems, lest we overlook some side-reactions which may be going on hand in hand with the main process, and may at times even play a major part, and thus account for the behavior of some animals which show neither great systemic disturbances when inoculated, nor produce antitoxins in quantity in response to such inoculations.

Reference to Horse 136 shows on the other hand that an animal possessing only a very weak protective serum primarily, may be perfectly capable of producing very high grade antitoxin. The experience of Meade Bolton confirms our own, for he writes[13] "that it would seem that the presence or absence of more or less antitoxin normally has no effect upon the ultimate production of artificial antitoxin by inoculation, but its presence enables the inoculation to be made with less risk to the animal." Bolton refers to these substances occurring in apparently normal sera as antitoxin, but we have spoken of them advisedly simply as protective or neutralizing substances, since their identity with the true diphtheric antitoxin is considered by some observers as at least doubtful.

Cobbett[14] has recently published a paper on the question whether the normal horse's serum contains diphtheric antitoxin. To solve the problem of the identity or non-identity of the protective substance with antitoxin he attacks it experimentally, basing his work and conclusions upon an acceptance of the toxin-toxoid composition of diph-

[13] Op. cit., p. 545.

[14] *Lancet*, 1899, ii, p. 332. Also, *Centralbl. f. Bakt.*, 1899, xxvi, p. 548.

theria-culture filtrates as set forth by Ehrlich. Testing the protective normal sera against the toxins in the manner indicated by Ehrlich, he finds that they act in all respects as typical antitoxins. He concludes that these experiments afford a proof of the identity of these substances. He does not, however, think that it follows that the antitoxin is a normal constituent of the serum of the normal horse, but rather that the diphtheric antitoxin present in the blood of certain men and horses is probably acquired, or, in the case of the young, inherited from the mother, by whom immunity has been acquired. "The presence of antitoxin in these animals cannot therefore," he claims, "be held to throw any light on the origin of antitoxin."

To enter into the question as to whether these protective substances are, in the accepted sense of the term, antitoxins or not, would lead us far afield into a discussion of the mechanism of and bases for the production of immunity in general, and of antitoxins in particular; yet we cannot but feel that, so far, the weight of evidence is on the side of those who hold that these substances are the same in composition as the so-called specific antitoxin, and further, that they are either normally present in the blood or capable of being produced under stress by the cells. By the term "specific" in describing antitoxin we mean derived from a personal infection, or an ancestral infection, near or remote, with the specific organism of diphtheria, or from an inoculation with its poisons.

Considering this subject briefly, we find in some horses, with no individual or ancestral history of infection with the diphtheria bacillus, a substance or substances which will protect test animals from multiple fatal doses of diphtheric poison; we find, also, we believe, both from our own experiments and from those of others, that the normal protective or neutralizing substances are globulins (or associated substances that cannot be differentiated from them) just as are the true antitoxic substances, which are formed during an infection or artificial immunization; and, furthermore, whether we accept or do not accept the views of Ehrlich[15] upon which Cobbett has based

[15] For a consideration of these views, based upon experimental work, see Park and Atkinson, *Journal of Experimental Medicine*, 1898, iii, p. 513.

his conclusions, still the fact remains that his experiments have shown that these protective substances in normal serum act toward the diphtheric toxins in precisely the same manner as do the true antitoxins.

If we look upon these facts as in any way proving the identity of the normal protective substances and specific antitoxin, then a disbelief in the antitoxins and the normal protective substances being normal physiological products or potential products of the cells of antitoxin-producing animals finds for its chief foundation a hypothetical past infection or past or present non-pathogenic association with the germs of diphtheria. Even if such infections or associations could be proven, the problem would simply be referred back in the process of evolution, but in nowise elucidated, since there must have been a point in history where no such infection or association had yet taken place, hence the fundamental question at issue is in regard to the bacterial (toxic) or cellular origin of the antitoxins.

Much weighty and convincing experimental evidence[16] has already been brought forward against such views as those of Fraser[17] "that the antitoxic or immunizing substances originate not from vital reactions upon constituents of the body, but from the toxins themselves, being produced by chemical changes in them, or being actually among their normal ingredients"; so that even assuming the presence of toxins, these would not account for the production of antitoxins, unless the latter were products of the cells, and simply increased in response to toxic action or stimulation.

Since we have, then, as far as we can determine, normal animals producing under normal conditions protective substances, which are, according to our tests, identical with the specific antitoxin produced in animals and man by disease or inoculation; and since, unless we assume in all cases a recent past infection or some constant source of toxin supply, neither of which can in most cases be shown to have a basis in fact, we are forced to turn to the animal economy for the supply of antitoxic substance. Hence we are inclined to look upon these substances as products of certain cells of the bodies of the animals in whose sera they are found, or as products which may be

[16] See Salomonsen and Madsen, *Annales de l'Institut Pasteur*, 1898, xii, p. 763.
[17] *Lancet*, 1898, ii, p. 247.

regarded as potential in the cells of these animals which show no normally present antitoxic bodies, but develop them in the presence of toxins. That this last supposition is not purely hypothetical is indicated, as shown above, by horses with practically no normally present protective bodies, but which develop antitoxic sera in response to inoculations.

That these protective substances subserve some other purpose in the normal physiological economy may or may not be true—but that they must have existed as normal physiological products or potentialities even in the first surviving animal attacked by the specific disease, providing cure resulted from the formation of an antitoxin, seems at least probable, if not undeniable.

It appears to us, therefore, that these substances or antitoxins, have in all likelihood had no absolute dependence upon or relation to toxin even remotely in the history of the race or individual, except as they may have been increased and adapted to the protection of the organism in the presence of an infection;[18] and hence that the normal protective substance and specific diphtheric antitoxin are one and the same substance or substances, which fluctuate within certain limits as do all other physiological products, according to the present need and condition and past history of the animal.

Conclusions.

The results of the foregoing experiments may be briefly summarized as follows:

The amount of antitoxic substance obtained by precipitation with magnesium sulphate from the blood-serum of the horse corresponds, as nearly as can be determined by the use of test guinea-pigs, in full to the protective power of the serum from which it is obtained, *i. e.* the precipitate from 1 cc. of serum will protect against the same amount of toxin as 1 cc. of the serum itself.

Equal amounts of the precipitates by magnesium sulphate from immunized and non-immunized horses act differently toward toxin; *i. e.* the proportion of protective substance to the precipitate from

[18] Cobbett has recently reported a conclusive observation of the natural occurrence in a horse of nasal and laryngeal diphtheria caused by Bacillus diphtheriæ (*Lancet*, Aug. 25, 1900, p. 573).—Editor.

non-immunized serum is exceedingly small as compared with the proportion of antitoxin to the precipitate from sera of immunized horses.

The average precipitate from the sera of immunized horses, as obtained by magnesium sulphate, is more abundant than the average precipitate from sera of non-immunized horses.

In the case of the same animal before and after immunization, the serum before immunization gives a less abundant precipitate with magnesium sulphate than the serum tested after immunization.

The proportion of increase per unit of antitoxic strength for the same or different horses is not constant. This may be due to an increase of inactive substances (in their relation to diphtheric toxin) or to imperfect methods of determination.

The precipitates obtained by magnesium sulphate give all the reactions recognized as characteristic of globulins, and as distinguishing them from other albuminous bodies. We are not warranted, then, in the present state of our knowledge, in considering any part of these precipitates as other than globulin. But it does seem warrantable to conclude, from the fact that the globulins of normal serum do not protect, or only in comparatively large amounts, against diphtheric toxin, that new globulins are formed, or rather greatly increased in the serum of immunized horses, and that these globulins protect against the toxin. These increased globulins and the inert globulins (which from obvious causes are a very variable factor) are both precipitated by magnesium sulphate.

Every animal has a physiological and pathological history more or less widely diverging from the normal, hence absolute conformity in the results obtained is not to be expected, at least with our present methods of differentiation.

We desire to express our sincere thanks to Dr. William H. Park, Assistant Director of the Research Laboratory, for the many courtesies shown us throughout the continuance of this work; and also to Dr. George P. Biggs for his valuable assistance in aiding us to procure suitable material for our experiments.

We are especially indebted to Prof. T. Mitchell Prudden of Columbia University for helpful suggestions during the preparation of this article.

THE FRACTIONAL PRECIPITATION OF THE GLOBULIN AND ALBUMIN OF NORMAL HORSE'S SERUM AND DIPHTHERIA ANTITOXIC SERUM, AND THE ANTITOXIC STRENGTH OF THE PRECIPITATES.

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In December, 1899, I published in this Journal[1] a preliminary note on the fractional precipitation of the globulin and albumin of normal horse's serum and diphtheric antitoxic serum. I now present the completed work, though there is still much which I cannot explain and which I have not as yet been able to work out.

The proteids of diphtheria antitoxic serum do not show any determinable differences chemically from those of the normal serum. The globulin precipitate[2]—which possesses the antitoxic power—increases in quantity but not necessarily proportionately to the antitoxic increase (see the preceding article, p. 47).

The method employed in the precipitation is as follows: 10 cc. of serum are pipetted into a beaker glass of suitable size and diluted up to a volume of about 50 cc. with water; the globulin is then removed by saturating the diluted serum with magnesium sulphate and filtering the precipitated globulin. The globulin precipitate is dissolved in water and again precipitated by magnesium sulphate, filtered off, and washed with saturated magnesium sulphate solution. It is thus freed from the albumin. The filtrates, which contain the albumin, are combined. The globulin derived from the 10 cc. of serum, is dissolved in about 200 cc.

[1] *Journal of Experimental Medicine*, 1899, iv, p. 649.

[2] The article of Brieger and Boer entitled "Ueber Antitoxine und Toxine" (*Zeitschr. f. Hygiene u. Infectionskrankh.*, 1896, xxi, p. 259), and the more recent work of Freund and Sternberg, entitled "Ueber Darstellung des Heilkörpers aus dem Diphtherieheilserum" (ibid., 1899, xxxi, p. 429) show that the antitoxin is not carried down mechanically by the globulin, but that there is a true precipitation of the antitoxic substance.

of distilled water and the solution is saturated with sodium chloride. The albuminous filtrate is also saturated with sodium chloride and set aside for the fractional precipitation of the albumin.

The globulin solution saturated with sodium chloride is allowed to stand over night at the room temperature (15-20° C.) before filtering off the precipitate which forms upon saturating. A shorter time may do quite as well but I have in all my experiments allowed it to stand over night in order to be sure of saturation. The precipitate after filtering is washed with the saturated sodium chloride solution, dissolved in distilled water and coagulated. 350-400 cc. of water is a convenient volume from which the coagulation of the R.° (room temperature) precipitate may be made by bringing the solution to a complete state of boiling and then adding weak acetic acid drop by drop until the liquid is faintly acid. The coagulated R.° globulin-precipitate is allowed to settle and is then filtered on a Gooch crucible, washed with hot distilled water, then with absolute alcohol, dried in the air-bath to constant weight, cooled in the desiccator over calcium chloride and weighed.

The beaker containing the filtrate from the R.° precipitate is placed in a water-bath, the temperature of which can be regulated easily, and the temperature is raised to 40° C., sodium chloride having been added to ensure saturation. At this temperature a turbidity makes its appearance and increases until 45° C. is reached. The solution is constantly stirred while the temperature is rising. The 40-45° C. precipitate can now be filtered off and the filtrate should run through the filter perfectly clear after 20 or 25 cc. have come through and have been poured back on the filter. If it continues to run through cloudy, the precipitation is not complete and the beaker containing the solution from which the precipitation is being made should be put back in the water-bath and the temperature run up to 45° C. again with constant stirring.

This precipitate also dissolves readily in water, and is coagulated, filtered, washed, dried and weighed as described. In the same manner one obtains a third turbidity at 49° C. and complete precipitation at 54° C.; a fourth turbidity at 57° C. and complete precipitation at 62° C. These four precipitates are soluble in water. Finally a fifth turbidity appears at 67° C. and there is complete precipitation at 72° C. The final precipitate is not entirely soluble in water but the few insoluble flocks are easily soluble in a little sodium hydroxide, and after neutralization with acetic acid their solution can be added to the general solution of this precipitate. The dissolved precipitates are coagulated, filtered, washed, dried and weighed as described.

TABLE I.

COMPARISON OF THE FRACTIONAL PRECIPITATES OF GLOBULIN OF NORMAL AND ANTITOXIC SERA, SHOWING ALSO THE LOSS OF GLOBULIN IN WEIGHT DURING THE PROCESS OF PRECIPITATION. 10 CC. OF SERUM WERE USED IN EACH CASE.

Temps. of Precip.	R° ppc.	40° ppc.	49° ppc.	57° ppc.	67° ppc.	Sum of Frac.	Total Glob.	Loss of Glob.
Horse No. 137 Normal Serum	.1457	Trace	.0482	.0746	.0071	.2756	.3235	.0479 gms.
137, bled 10/20 '99, 1200 units per cc.	.0648	.1026	.1247	.1899	.3038	.7856	.8292	.0436 gms.
137, bled 10/20 '99, 1200 units per cc.	.4419	.0397	.2970	.0362	Trace	.8144	.8292	.0148 gms.
137, bled 11/9 '99, 650 units per cc.	.1729	.1086	.1253	.0950	.0124	.5142	.5934	.0792 gms.
137, bled 11/14 '99, 600 units per cc.	.0613	.0992	.1007	.1486	.0218	.4316	.5116	.0800 gms.
137, bled 11/18 '99, 400 units per cc.	.2200	.0733	.0842	.0679	Trace	.4454	.4743	.0289 gms.
" "	.2225	.0724	.1308	.0227	Trace	.4448	.4743	.0295 gms.
" "	.0853	.0637	.0846	.2038	.0188	.4562	.4743	.0181 gms.
" "	.1691	.0446	.0713	.1386	Trace	.4236	.4743	.0507 gms.
Horse No. 135, Normal Serum.	.1154	Trace	.0373	.0749	.0399	.2375	.3864	.1489 gms.
135, bled 10/26 '99 500 units per cc.	.2209	.1408	.1944	.0714	.0149	.6424	.8987	.2563 gms.
Horse No. 136, Normal Serum.	.1005	.0056	.0597	.1450	.0058	.3166	.3536	.0370 gms.
136, bled 10/20 '99, 600 units per cc.	.1277	.0530	.1249	.1919	.1118	.6093	.7389	.1296 gms.
Horse No. 133, Normal Serum.	.0485	.0124	.0378	.0731	.1473	.3191	.3727	.0536 gms.
133, bled 9/18 '99. 400 units per cc.	.1376	.0604	.0995	.1304	.2600	.6879	.7782	.0903 gms.

The final filtrate from the 67-72° C. precipitate fails to give the biuret reaction, and on boiling and subsequent addition of a little acetic acid shows no turbidity. These reactions are common to the globulin of normal and diphtheric antitoxic globulin. Duplicate analyses give frequently different results for corresponding temperatures (see Table I). These differences cannot be accounted for by incomplete precipitation, for if, for any temperature the precipitation was found to be incomplete, the solution was again raised in temperature to the proper degree and the globulin was filtered out and added in its proper place. They could be accounted for by considering each precipitate as caused by the formation of a globulin salt. The amount of the precipitate at any given precipitation tempera-

ture would then depend upon the amount of the compound formed. At the close of the paper I shall consider more fully this hypothesis.

The globulin suffers a loss during the separation (see Table I), and I have found nitrogen in the 67-72° C. filtrates in varying quantities. In order to determine whether the action of sodium chloride had a destructive action on the globulin, three determinations were made by coagulating the globulin from 10 cc. of serum for each experiment.

1. From the watery solution containing some magnesium sulphate which remained with the globulin when it was separated from the albumin.
2. From a one-half saturated sodium chloride solution.
3. From a saturated sodium chloride solution.

Table II gives the results, which show that sodium chloride does not exercise a destructive action on the globulin.[3]

TABLE II.

COMPARISON OF COAGULATION WEIGHTS OF GLOBULIN, EACH FROM 10 cc. OF SERUM, FROM A MAGNESIUM SULPHATE SOLUTION AND FROM SODIUM CHLORIDE SOLUTIONS.

Horse 133.
Normal serum.
Weak $MgSO_4$ solution = .3727 grms. of coagulated globulin.
One-half saturated NaCl solution = .3656 grms. of coagulated globulin.
Saturated NaCl solution = .3648 grms. of coagulated globulin.

The globulin fractions from antitoxic serum each contain antitoxin and the final filtrate is free from antitoxin. Some of the antitoxin is always destroyed by the process of precipitation. In this reaction the relation of "diphtheria antitoxin" to globulin is possibly still further shown, the loss in antitoxic power and the loss in weight of the globulin being at the same time and by the same cause.

Table III gives the analyses of a serum containing 400 units per each cubic centimetre which during the process of precipitation lost about 46% of its antitoxic strength.

[3] Solutions 2 and 3 also contained a little magnesium sulphate which remained with the globulin when it was separated from the albumin.

TABLE III.

THE RELATION OF ANTITOXIC STRENGTH TO WEIGHT OF FRACTIONAL GLOBULIN PRECIPITATES PER CUBIC CENTIMETRE.

Serum of Horse 133, bled September 18, 1899—400 units per cc.

Temperature of precipitation.	R° ppc.	40° ppc.	49° ppc.	57° ppc.	57° filt.	67° ppc.	Sum of frac.	Original am't of globulin per cc.	Loss of globulin.
Weight of globulin precipitates in grms.	.0457	.0036	.0132	.0078	.0103	.0011	.0714	.0778	.0064
Antitoxic strength of precipitates in units.	100 to 115	10 to 15	50 to 75	25 to 50	40	10 to 15	195 to 237	Original strength 400 units per cc.	Mean loss of antitoxic strength 184 units = 46%.

This table also shows that antitoxic power is lost during the heating process, while the proteid may be scarcely affected in weight, for if the globulin from the 57° filtrate is substituted for the globulin obtained from this filtrate, *i. e.* the 67° ppc., we have a sum of the fractions .0028 grms. greater in weight than the total globulin. That is, the sum of the fractions and the total globulin are practically the same, and we have a loss of 40% of antitoxic power. We may explain this by considering the antitoxic molecule rearranged by the lower temperature during the process of precipitation without the destruction of the coagulable properties of the proteid.

It will be seen by Table III that there is only a general relationship between the number of antitoxin units and the corresponding weight of globulin.

The destruction of the globulin and antitoxin by the process of precipitation is directly shown by the difference in globulin and antitoxin found in the 57° C. filtrate and the amount found in the precipitate obtained from it.

These reactions of normal and antitoxic globulins toward the salt precipitants, coupled with the fact that normal horse's serum is found to possess decided antitoxic properties against diphtheria toxin and that this antitoxin in normal horse's serum is also found in the globulins, compels us, I believe, to place diphtheria antitoxin among the globulins. The non-correspondence of change in globulin weight to antitoxic increase and decrease, except in a very general way, indi-

cates in immunized as well as in normal serum the presence of other forms of globulin besides the antitoxic, all of which are affected by the group reagents and coagulated by heat in the same way.

The fractional separation of the albumin (Table IV) is carried out as described for the globulin.

The albuminous filtrate which has been saturated with sodium chloride is poured off from the mass of salts[4] at the bottom of the beaker. The salts are washed with a saturated sodium chloride solution and the washings are added to the main portion of the solution. Some albumin may be held back in the crystals, and in order not to have too large a volume to work with, I have not washed very carefully but have dissolved the crystals and coagulated the proteid which they held. This is afterwards distributed proportionately among the various precipitates. The first precipitate of the albumin makes its appearance at 56° C. and may be filtered off at 61° C. This precipitate is soluble in water. The second precipitate appears at 67° C. and may be filtered off at 72° C. This precipitate is partially soluble, a portion being coagulated and needing weak sodium hydroxide for its solution. The third and fourth precipitates are not soluble, but are coagulated. They are thrown out of solution at 73-76° C. and 77-81° C. respectively. They are at best only slight and in some cases only represented by traces. The final filtrate is free from coagulable proteids and fails to give the biuret reaction. As in the globulin so in the albumin, some of the proteid is lost during the separation.

Dr. Thomas B. Osborne of the Connecticut State Agricultural Station has shown that certain vegetable proteids form definite compounds with

[4] In the preliminary communication, I stated that a double salt was formed by saturating the magnesium sulphate filtrate, containing the albumin, with sodium chloride. The statement is incorrect. Almost every analysis showed the salt to be sodium sulphate. The following are 3 selected analyses of the salt:

No. 1. 1st crystallization.			No. 2. 1st crystallization.	
Mg =	9.45%		Mg =	4.54%
SO_4 =	38.26%		No other constituent quantitatively determined. Cl present.	
H_2O =	51.89%	determined by loss in weight.		
	99.60%			

No. 3. Recrystallized four times.		
H_2O =	56.29%	Impurity of Cl and Mg in 1st crystallization. Cl persisted until fourth crystallization.
SO_4 =	29.81%	
Na =	13.9%	by difference
	100.00	

mineral acids. Thus, he has separated edestin[5] mono- and bihydrochlorate. The nitrate, phosphate, sulphate and acetate of edestin are indicated by the reaction. The behavior of the animal globulins and albumins may find their explanation in the same way. The individuals of the globulin group may form sulphates insoluble in saturated magnesium sulphate solution or may form double salts (globulin magnesium sulphate) insoluble in saturated magnesium sulphate solutions. If we saturate the "globulin solution" with sodium chloride at room temperature a precipitate separates out. The precipitate may be accounted for by considering the chlorine or sodium chloride as uniting with or effecting a double decomposition with the globulin sulphate or globulin magnesium sulphate, and the formation of either a simple globulin chloride or double salt of globulin sulphate and chloride, or, perhaps, a complex salt containing globulin magnesium sodium chloride and sulphate. The precipitations at the other temperatures could be accounted for by the same reactions.

TABLE IV.

COMPARISON OF THE FRACTIONAL PRECIPITATES OF ALBUMIN OF NORMAL AND ANTITOXIC SERA; SHOWING ALSO THE LOSS OF ALBUMIN IN WEIGHT DURING THE PROCESS OF PRECIPITATION.

10 cc. of serum were used in each case.

Temperature of precipitation.	56° ppc.	67° ppc.	73° ppc.	78° ppc.	Sum of fractions.	Total albumin.	Loss of albumin.
Horse No. 137, Normal Serum	.2259	Traces for 67°, 73° + 78°			.2259	.3127	.0868 grms.
137, bled 10/20 '99, 1200 units per cc.	.1558	.0180	Traces for 73° + 78°		.1738	.2046	.0308 grms.
137, bled 11/9 '99, 650 units per cc.	.1987	.0204	Traces for 73° + 78°		.2191	.2727	.0536 grms.
137, bled 11/14 '99, 600 units per cc.	.1791	.0351	Traces for 73° + 78°		.2142	.2938	.0796 grms.
137, bled 11/18 '99, 400 units per cc.	.1253	.0457	73° + 78° = .0080		.1790	.3133	.1343 grms.
Horse No. 135, Normal Serum	.0304	.0672	.0074	.0061	.1111	.3680	.2569
135, bled 10/26 '99, 500 units per cc.	.1520	.0188	73° + 78° = .0133		.1841	.2341	.0500
Horse No. 136, Normal Serum	.2149	.0428	73° + 78° = .0078		.2655	.3618	.0963
136, bled 10/20 '99, 600 units per cc.	.0290	.1193	73° + 78° = .0612		.2095	.2715	.0620
Horse No. 133, Normal Serum	.2438	.0986	73° + 78° = .0362		.3786	.4146	.0360
133, bled 9/18 '99, 400 units per cc.	.0877	.0407	73° + 78° = .0266		.1550	.2101	.0551

[5] *Jour. of the American Chem. Soc.*, 1899, xxi, No. 6, June.

The precipitation of the albumin by means of sodium chloride could be accounted for in the same manner. At 73° C. and 77° C. we have coagulation and these portions of albumin are therefore beyond this hypothesis.

The presence of the SO_4 is necessary for these fractional precipitations of the globulin and albumin. According to standard text-books some of the globulin can be precipitated from the serum by saturating the serum with sodium chloride at room temperature. I have obtained another precipitate from the room temperature filtrate by saturating with sodium chloride at 56° C. These precipitates are slight however. In a serum containing 600 units of antitoxin per cc. I obtained a precipitate containing only 10 units per cc. by saturating with sodium chloride at room temperature. Halliburton[6] states that serum albumin is soluble in saturated sodium chloride and magnesium sulphate solutions, yet when the serum has been treated with magnesium sulphate, one can precipitate fractionally the globulin from its watery solution, and the albumin from its magnesium sulphate solution by saturating these solutions with sodium chloride and raising the temperature.

If it be true that we have a series of compounds of globulin and albumin in the described reactions it would account for the differences in the quantities of globulin obtained at corresponding temperatures from duplicate samples, the amount of the precipitate depending upon quantities of the compound formed.

I hope to be able to show later in what form these precipitates of globulin and albumin occur, whether as pure proteids, simple halogen salts, double salts or complex salts.

CONCLUSIONS.

1. The globulins of both normal and diphtheria antitoxic serum exhibit chemically toward reagents the same reactions, being precipitated by magnesium sulphate and split up into fractions in precisely the same way.

2. All of the diphtheric antitoxic power of both normal and immunized serum is always carried by the globulin and its fractional precipitates.

3. During the fractional precipitation of the serum globulin of horses immunized from diphtheria toxin and horses not immunized

[6] Halliburton, *Text-book of Chemical Physiology and Pathology*, pp. 127 and 236–237.

from diphtheria toxin, some of the globulin is lost, likewise at the same time some of the antitoxic power of the globulin of the immunized serum is lost.[1]

4. These reactions, considered in connection with the fact that different observers as well as we ourselves have found diphtheric antitoxic power in normal horse's serum and that this antitoxin separates with the globulin, strongly incline us to consider "diphtheria antitoxin" a form of globulin.

5. The reactions of globulin, previously separated from the serum by magnesium sulphate, with sodium chloride lead one to think that there is a formation of globulin salts.

6. Since serum albumin in a magnesium sulphate solution gives fractional precipitates at definite temperatures, it seems not improbable that the albumin is precipitated in the form of albumin salts.

I take this opportunity of expressing to Dr. William H. Park, Assistant Director to this laboratory and Dr. Alexander Lambert, Assistant Bacteriologist, my appreciation of their suggestions and kind criticisms during my work.

[1] On the addition of the fractions of antitoxin tested in guinea pigs, and the addition of the fractions of globulin determined by weighing the coagulated globulin, we find that both have lost, but that the antitoxin has been affected especially by the higher temperature.

REFRACTORY SUBCUTANEOUS ABSCESSES CAUSED BY SPOROTHRIX SCHENCKII. A NEW PATHOGENIC FUNGUS.[1]

By LUDVIG HEKTOEN, M. D., Chicago, and C. F. PERKINS, M. D., Shenandoah, Iowa.

(*From the Pathological Laboratory of the Rush Medical College.*)

Plates II and III.

In the Bulletin of the Johns Hopkins Hospital for December, 1898, there appeared an article by B. R. Schenck "On Refractory Subcutaneous Abscesses caused by a Fungus possibly related to the Sporotricha." "The primary point of infection was on the index finger, whence it extended up the radial side of the arm, following the lymph channels, and giving rise to several circumscribed indurations, which were in part broken down and ulcerated." This infection proved very refractory to treatment. The organism supposed to cause the lesions in this case was obtained in three cultures from two different foci of the disease, twice in pure culture.

Schenck carefully describes the cultural characteristics, the morphology and development and the results of the inoculations of this organism which Dr. Erwin F. Smith, of the United States Department of Agriculture at Washington, tentatively assigns to the genus *Sporotrichum*.

During the last few months we have had occasion to study a case presenting similar refractory subcutaneous abscesses from which an organism has been isolated which is identical in the essential details with the one described by Schenck.

[1] Presented at the Fifteenth Annual Meeting of the Association of American Physicians, held in Washington, May 1–3, 1900. A brief statement concerning the organism was also made by Professor Jordan for the authors at the meeting of the Society of American Bacteriologists in New Haven, Conn., Dec. 28, 1899.

Clinical History (Dr. Perkins):—"On March 16, 1899, Charlie C., aged five years, was brought to my office by his parents, suffering with a sore upon his left index finger, concerning which I elicited the following history: Ten days before he had been using a hammer and had struck himself denuding a surface as large as a split pea on the dorsal surface and over the second joint. This abrasion did not heal up, as his father thought it should, and he applied some 'verdigris salve' to the injury. At present the finger, from the first to the third joints, is swollen to twice its original size, presenting in the centre a deep, well defined, sharp, undermined ulceration, the size of a ten-cent piece. The base of the ulceration is rough and covered with grayish-looking pus. This, when sponged away, leaves a bright red surface; the ulcer extends through the whole thickness of the skin. Surrounding the ulcer over about one-half of the infiltrated area are a large number of vesicles and a few pustules. The dorsal surface of the hand and the extensor surface of the forearm present a chain of swollen lymphatics along which are about twenty nodules the size of a small pea to a large hazel nut. There is no evidence of suppuration in any of them at this time. The little patient does not complain of much pain, either in the finger or arm. The hand is cleaned with green soap and washed with sublimate solution 1 to 2000; the surface of the ulceration mopped over with 95% carbolic acid, powdered boracic acid dusted on, and bichloride gauze and borated cotton applied.

"Subsequently the dressing was changed every second day for ten days and reapplied as at first. At the end of this time ulceration has increased to nearly twice its original size. The nodules are somewhat larger and are getting tender though I cannot detect pus, either by fluctuation or aspiration. On March 28 I began using iodoform as a dressing, but met with no better results. Do what I would, I could not get the finger to improve in the least. The vesicles had formed pustules and the epidermis over nearly all of the infiltrated area had loosened, leaving a raw oozing surface.

"During the first ten days under my care the child seemed to feel reasonably well, but he now began to develop a little fever, and for the next month the temperature ranged up to 100.5° F. There developed about April 1 an annoying and persistent cough and coryza. Early in April the swollen lymphatics began to suppurate. I opened and curetted eleven abscesses in the next week. Some contained not more than thirty minims, others probably four drams of pus. Upon opening one large abscess about the middle of the forearm, I was surprised to

see it drain another smaller one four inches from the incision. This was refilled with the irrigating solution and opened. The fistulous tract was irrigated and a swab with carbolic acid upon it was passed through. The pus from the abscesses was of a mahogany color, thick and tenacious.

"Up to this time (May 25, 1899), I have opened twenty-one abscesses, four have opened spontaneously and still there are more to follow.

"The lymphatic glands in the axilla and neck on the left side have been inflamed, but are smaller now and bid fair to return to their normal size without suppurating. The abscesses which have been opened have shown considerable destruction of the fascia, intermuscular septa and skin.

"Suspecting farcy and with the expectation of having my opinion confirmed, I inoculated two tubes with a quantity of pus from an uncontaminated abscess and mailed them May 2 to Prof. L. Hektoen for examination. At his request, I again sent him tubes about May 9 or 10.

"The family being poor, I have had the mother dress the arm part the time. I once saw her doing this when she had a cut over one-half inch long on the thumb. The quantity of pus from the sores was large at this particular time and it seems impossible that she did not get germs into the wound. This fact and that the patient has mingled with several other children in the family have convinced me that the danger of infection is not very great.

"Internal treatment has varied somewhat: Cough mixtures for two or three weeks; calcium sulphide in $\frac{1}{4}$ grain doses; corrosive sublimate 1/100 grain with quinine sulphate 1 grain. The last named associated with wet dressings of 1 to 100 carbolic acid seemed to do the most good. Two small photographs were taken before any of the abscesses had been opened. A larger one was made May 25, 1899, which shows the present condition very well (Plate II, Fig. 1). The boy's condition has improved very materially and my opinion is that he will fully recover in another month" (which proved to be the case).

Bacteriological Examination.—The glycerine-agar tubes, inoculated by Dr. Perkins on May 2 and again on May 10, all developed pure cultures of the organism in question. After about 6 days, the last four being in the incubator at 37° C., there appeared several greyish-white, raised, irregular colonies, about as large as a pin's head, confined exclusively to the area of the surface of the medium covered by the bloody exudate. In one tube similar but smaller growths

appeared in a small quantity of exudate accidentally smeared upon the glass. The colonies were rather dense and viscid; considerable increase took place and wrinkled masses formed. Repeated subcultures upon various media show the following cultural characteristics:

Agar (Plate II, Figs. 2-4).—At the end of 24 hours in the incubator there is some growth of a greyish color and granular in appearance along the streak on plain and glycerine agar. After 48 hours the growth appears as a delicate, slightly raised, whitish line with symmetrical feathery fringes and some hairy downgrowth into the substance of the agar. At the end of 72 hours the growth assumes the form of a band with numerous transverse wrinkles; in a couple of days more the surface becomes more markedly corrugated and looks like the chains of mountains on a map. About the 7th day the growth, which has increased some in thickness, becomes light brownish in color, the margins being smooth and wavy, marked by shallow transverse grooves. Still later the growth becomes distinctly and even dark brown, the surface wrinkled and velvety, in some cases covered by a very delicate fuzz. The medium becomes slightly brownish.

On wort-agar a thick yellowish, wrinkled membrane develops, extending some distance upon the sides of the tube. This, or glucose-agar, is one of the best media for rapid growth.

The individual colonies upon glycerine-agar plates appear at the end of 2 to 4 days as irregular, greyish-white dots, 0.4-1 mm. in diameter, resembling somewhat minute flakes of snow. Under the microscope they are made of a central network of threads which at the periphery grow outward in a radiating manner and become tipped with small clusters of minute dots; similar dots (conidia) also appear along the sides of the outgrowing threads (Plate III, Fig. 5).

Individual colonies on agar slants, after three or four days, appear as minute shreds which gradually develop into round, circumscribed, raised, somewhat pearly masses which send fluffy prolongations into the medium; later the surface of the spreading colony becomes raised into irregular wrinkles and eventually a brownish color generally appears.

The early surface-growth in the glucose agar stab presents a whitish, heaped up centre and delicate radiating margins; with time a uniform growth develops along the stab with fine, thickly set, lateral branches. No fermentation.

In one or two instances streak cultures upon agar have resulted in a flat, granular, sparse growth only, consisting almost wholly of round and

oblong budding bodies. Subcultures from such growth give rise to characteristic folded and wrinkled membranes.

Blood serum.—In 48 hours small colonies appear which are covered by a white frosting; the growth increases slowly but presents no special characteristics. There is no liquefaction.

Gelatin.—The deep growth is confined to the upper end of the stab; it increases slowly and sends out lateral branches which are longest immediately underneath the surface which becomes covered by a flat, spreading layer. At the end of about 6 to 7 days a slight liquefaction is apparent; in about 14 to 18 days later the liquefaction is nearly complete, the surface is covered by a dense membrane which has a tendency to sink down into the clear liquid.

Wort-gelatin, acid gelatin and 4% glycerine gelatin are more favorable for growth, and liquefaction takes place earlier; on shaking, the thick surface layer falls to the bottom, afterwards a new layer forms on the surface and delicate threads may be seen growing vertically along the space of the tube. The liquid gelatin remains clear.

Potato.—Twenty-four-hour cultures show a slight brownish-grey or yellowish nodular growth which increases quite rapidly, becomes raised and wrinkled, the surface presenting smaller and larger districts of a white, frosted appearance. The older growths become discolored at the same time as the potato is darkened.

Milk.—Litmus milk is not changed in color and not coagulated; but slight growth takes place.

Bouillon.—The growth is fairly abundant; little tufts or shreddy masses form which settle at the bottom or cling to the sides of the tube; a white, crumbling surface-film is occasionally observed; no change in reaction. The rate of growth seems about equal in plain, glucose, and glycerine-bouillon.

Vegetable infusions.—The fungus grows in hay, turnip, carrot and potato infusions as a whitish, flocculent precipitate, the fluid remaining clear.

Hydrant water.—A very slight growth?

Gasperini's Starch.—A flat, slightly raised, greyish-white, hard layer forms rather slowly.

Fermentation Test.—Glucose, lactose and saccharose bouillon, prepared according to the method of Theobald Smith,[2] shows a characteristic growth in the aërobic bulb, the anaërobic tube is unchanged and there is no gas formed.

[2] *Journal of Experimental Medicine*, 1897, ii, p. 546.

Anaërobiosis.—There is no growth in the tubes in Buchner's jars.

Temperature.—The optimum temperature for development would seem to be about 37° C.; good growth occurs at the room temperature but at a much slower rate. No growth occurs at a temperature just above the freezing point, but cultures kept at this temperature for 5 weeks remain alive and give rise to vigorous new growths on reinoculation.

Thermal Death-Point.—The organism is killed by an exposure to 60° C. for 4½ minutes; a slight growth occurred after an exposure to this temperature for 4 minutes. No growth could be obtained after exposure to 59° for 10 or 5 minutes, to 61° and 62° for 2 minutes, to 65° for 1½ minutes.

Morphology.—Cover-slips from the cultures, stained with methylene blue, show masses of more or less parallel, or tangled, straight or curved, unevenly stained, rather thick threads with rather infrequent true side-branches. Interspersed among the threads lie numbers of ovate or apiculate bodies, from 3 to 5 μ in their longest diameter. In the stained preparations no definite connection is to be made out between the majority of the bodies and the threads. In some preparations the bodies predominate. Occasionally bodies are seen connected with a thread by a small pedicle. The threads seem thinner when stained with Gram's method which gives them a light violet, granular appearance with irregular clear spaces. Gram's method stains the spore-like bodies a deep blue; occasionally there is an unstained area in their interior. Some spores appear to be growing out to form short threads.

In the unstained preparations and in hanging drop cultures of bouillon and gelatin, the threads of the mycelium are seen to be doubly contoured; the protoplasm is somewhat granular and interrupted at fairly regular intervals by transverse septa; the diameter of the threads varies somewhat, the average being about 2 μ; the branches are not frequent and do not bear any fixed relations to the septa.

In the hanging drop cultures the relations of the conidia to the mycelium are very nicely shown. The spore-bearing branches, which grow out in a radiating manner from the central felt-work, are commonly tipped by a cluster of from three to six or more conidia which, in the case of the larger clusters, are attached by the smaller end to the slightly expanded extremity of the branch. Similar ovate buds also arise from the sides of the hyphæ at shorter or longer intervals.

The spores are also doubly contoured and granular, resembling very much yeast cells.

These various features are well shown in the photographs of the growing hanging-drop cultures (Plate III, Figs. 6 and 7).

The attachment, by means of short pedicles, of the spores to the threads is very easily severed as shown by the difficulty in obtaining stained preparations with the spores in situ.

Development.—When placed in the hanging drop the conidia grow out into one or more straight germ tubes which spring from either, or both ends, or from the sides. These embryonal threads again give rise to lateral or terminal buds, which in all particulars resemble the spores and some of which form branching spore-producing threads, so that in the early stages very peculiar looking bodies are produced.

Histological Examination.—A small bit of skin including part of the wall of an abscess was excised by Dr. Perkins and sent to me in alcohol. The sections show some thickening of the epithelial layer; there are rather broad interpapillary downgrowths. On the cutaneous surface there is much horny material, altered red blood-globules, detritus and polymorphonuclear leukocytes often collected into dense foci within and upon the horny layer. The epithelium and the cutis show many leukocytes, with long drawn-out thread-like and contorted nuclei, apparently going in various directions and also aggregated into small groups. The vessels of the cutis are congested.

Along one side and part of the lower or deep surface of the section there is considerable bloody and leukocytic exudate; the adjacent tissue is infiltrated with leukocytes with greatly elongated nuclei which might be taken for fragments of fungus threads.

In sections stained by the Weigert and Gram methods are short threads which cannot be otherwise distinguished from the leukocytic nuclei mentioned. With this possible exception no micro-organisms are seen.

Animal Experiments.—*Rabbits.*—I-II. Injections of 2 cc. of a 24-hour bouillon culture into the abdominal cavity and into the ear vein of two large rabbits respectively produced no symptoms in 8 weeks.

III. A few drops of a bouillon culture inserted into the anterior chambers of the eyes of a small rabbit in 3 days produced a moderate whitish exudate, and a few, pin-head sized, white clumps formed which persisted for 2 days after which rapid recovery took place.

Guinea-pigs.—I-III. Intraperitoneal injections of 2 cc. of a suspension in bouillon did not produce any symptoms or changes after 6 weeks in the case of three animals.

IV. May 17 I injected a guinea-pig with 2 cc. into the subcutaneous

tissue over the abdomen. A number of small, pea-sized, firm nodules developed about the site of the injection. The animal died May 29. The subcutaneous nodules were small abscesses; in the pus were numerous oval and oblong bodies staining irregularly with methylene-blue and with Gram's method. No other organisms were found and pure growths of the sporothrix developed in the cultures from the pus. The internal organs were sterile and of normal histological structure.

V. A guinea-pig inoculated subcutaneously with 2 cc. July 25 died Aug. 18. There were no local changes visible and cultures from the internal organs remained sterile. At this time a number of guinea-pigs in the laboratory were dying from unknown causes.

Dogs.—I. Intravenous injection of 1 cc. of a suspension in bouillon did not produce any symptoms; all organs appeared to be normal and proved to be sterile when the animal was killed on the 34th day.

II. Two cc. of a bouillon suspension was injected into the subcutaneous tissue of a large, female dog. In a few days a small swelling appeared which was quite tender; a diffuse slightly tender induration remained for some time.

Twenty-eight days after the first injection 2 cc. were again inoculated under the skin of the thigh followed by the development of a small, fluctuating swelling. There was no enlargement of the corresponding inguinal glands. The animal was killed on the 15th day. The internal organs were normal and sterile. At the site of the first injection was an area of scar tissue which on microscopic examination showed islands of marked round cell infiltration; no organisms could be demonstrated in the sections and the cultures from the scar tissue remained sterile.

At the site of the second injection was a small, soft, whitish spot or cavity containing a gelatinous material, smears and cultures from which showed the organisms injected to be present in fair numbers. The sections, stained by Gram's method, showed organisms in fair numbers among the leukocytes in the little cavity which was enclosed in a recent fibrous tissue. The organisms resembled somewhat the conidia of the fungus, stained irregularly, and varied in size, being oval or oblong and from 2 to 3 or 4 μ in length.

III. Intraperitoneal injection of 2 cc. of a bouillon suspension did not produce any lesions, the organs being healthy and sterile, when the animal was killed on the 26th day.

White Rats.—I. Subcutaneous injection of 2 cc. of a bouillon culture did not seem to have any effect, the animal remaining well and fat.

II. About two months after the intraperitoneal injection of 2 cc. of

a bouillon culture, during which time the animal remained well, it was noticed that the scrotum was very large and dragged on the ground, interfering with the animal's movements; the hind legs were œdematous. Killed by chloroform. The larger part of the small intestine and omentum occupied the two sides of the scrotum. The peritoneum over the lower third of the abdominal cavity and lining the scrotal cavities, was covered with numerous yellowish, tuberculiform nodules, arranged now singly, now in groups or clusters. On incision each nodule was found to contain a quantity of yellowish-grey, viscid purulent material composed of leukocytes and innumerable, irregularly stained (Gram), oblong and oval conidia from 2 to 4 μ in length. Many presented a transversely striated appearance, in others there was a clear spot near one end. There were no threads present in the pus. The other organs seemed normal; cultures from them and from the heart's blood remained sterile; no organisms were present in any of the smears. The cultures from the abdominal nodules gave rise to innumerable characteristic colonies solely of the organisms injected.

The sections from the peritoneal nodules show small cavities enclosed in recent fibrous tissue; these cavities contain polymorphonuclear leukocytes, nuclear detritus, and the conidia of the organism in large numbers (Plate III, Fig. 8), most numerously just around the inside of the wall; here they are scattered about singly or arranged in groups; nearly all are extracellular but intracellular groups occur; if the Gram stained sections be allowed to remain in alcohol for a little longer time than that just sufficient for decolorization then the organisms lose their stain largely and may appear as cocci or short, thick bacilli of various sizes. The organisms cannot be distinguished in the hematoxylin and eosin specimens. Giant cells are not seen in the interior of the abscesses but in the recent fibrous tissue of the walls lies an occasional multinuclear cell of the tubercular type. In a few places the appearances indicate beginning abscesses; the first effect of the organism is a necrosis of the tissue followed by cell accumulation; it cannot be made out clearly just how the organisms are carried, or go, from an older to a recent focus; the presence of bodies within cells, especially leukocytes, indicates that they may be transported by wandering cells; a few single bodies are found scattered about in the tissue outside of the abscesses.

Grey mice.—I. Intraperitoneal injection of .5 cc. of a bouillon suspension produced death in 2 days. There was a purulent peritonitis, the organism being recovered from the exudate in pure growth. The internal organs were sterile and normal.

II. Subcutaneous injection of .5 cc. was followed by death after 2 days. There were no special changes and the cultures remained sterile.

III. Subcutaneous injection of .5 cc. was followed by evident symptoms of illness ending in apparent recovery. The mouse died 18 days afterwards, but on account of beginning decomposition no cultures were made.

IV-V-VI. Subcutaneous injection of .5 cc. Twenty days later one died, but the body was eaten by one of the survivors. This died two days later; there was some induration about the site of the inoculation, the tissues being dry and shrunken. The cultures and smear preparations were negative. About this time the third mouse showed considerable shrinking about the injection—at the root of the tail; a small, dry ulcer formed, one of the extremities appeared fixed and rigid so that the animal limped a good deal. It became thin and died four weeks after the inoculation. The tissues about the ulcer were firm and fibrous; underneath the skin over the upper part of the right thigh and extending upon the under surface of the abdomen was a collection of whitish, viscid, caseous pus which contained oval and oblong bodies in large numbers, and growing readily on glucose agar. The internal organs appeared normal both on gross and microscopic examination and the smears from them did not show any organisms.

White and tame mice.—Four white mice were inoculated with .5 cc. of a 48-hour old bouillon culture under the skin at the root of the tail. All reacted in about the same way. None died soon after the injection. In the 2nd or 3rd week they appeared to be getting thin. Beginning at the root of the tail and extending for a variable distance over the back there now formed a hairless, red area with an uneven surface; scattered over it were occasional yellow spots. The posterior part of the body seemed more or less shrunken, the hind legs stiff, one often more so than the other; in one mouse the left posterior extremity seemed fixed and drawn up against the body. I killed one with chloroform on the 23rd day, one died spontaneously on the 26th, one on the 35th, and the fourth on the 40th day. In all there was found some undermining of the edges of the ulcerated surfaces described; the ulcer was superficial and did not extend into the muscles; at some points the tissues about the margins were firm and fibrous; where the skin was undermined small quantities of semisolid cheesy material were found; sometimes such collections would extend for a considerable distance in the subcutaneous tissue; this material was composed largely of leukocytes and among them were found, in varying numbers, organisms like the conidia of the

fungus; in one animal there were numerous cocci also; in this case the cultures were mixed, most of the colonies being cocci, but quite a few colonies of the fungus developed. In all cases the internal organs were normal with the exception of a possible enlargement of the abdominal lymph glands; no organisms were found in the smears and the cultures remained sterile from the organs, which, including the lymph glands, were fixed in Zenker's fluid; the sections showed no changes and organisms were not found in those stained by Gram's method or otherwise.

In order to study the evolution of the anatomical changes produced by this organism, four tame mice were injected simultaneously into the abdomen with 1 cc. of a bouillon suspension of the same culture and killed at successive intervals of one week each. At the end of the first week a number of small nodules the size of a pin's head had developed near the site of the injection and in the omentum near the spleen which was adherent to the stomach; the retroperitoneal glands were not enlarged; the cultures from the nodules remained sterile; the sections (Weigert, hæmatoxylin, etc.) of the nodules show accumulations of cells with marked nuclear fragmentation and the formation of fibrin especially at the periphery; scattered about are oval and lanceolate organisms often in groups of 4-6 or more.

At the end of the second week there were found nodules, a little larger, scattered principally over the peritoneum of the scrotal pouches. Cultures successful. Sections show the nodules to consist largely of a cellular granulation-tissue with smaller foci of marked nuclear disintegration containing a much larger number of organisms than the nodules of a week's duration.

The third mouse died spontaneously on the 20th day after the injection. There was a small ulcer over the lower part of the abdomen, and innumerable yellowish areas and more diffuse flat thickenings with distinct spots of viscid softening over all parts of the peritoneum, the under surface of the liver, etc. Cultures successful. The sections show the tissue to be more fibrous while the foci of softening contain granular and amorphous detritus, small nuclear clumps and innumerable organisms often aggregated into heaps which appear as blue spots visible to the naked eye. In none of these nor in any of the animals were fungous threads found in the lesions.

The fourth died spontaneously and was eaten in the abdomen by another mouse in the same cage.

There can therefore be no question but that the spore-forms multiply in the lesions they produce in susceptible animals.

Pigeons.—Subcutaneous injections in two white pigeons of 2 cc. of bouillon cultures did not produce any lesions or symptoms in 3 weeks.

There can be no doubt in regard to the identity of the organism described by Schenck and the one described in this article. The two correspond morphologically and culturally and their pathogenic actions in animals are the same with the exception of a few easily explainable, insignificant differences. Schenck observed a general infection after subcutaneous injections in mice; as far as I know this did not occur in any of the animals that I used. And I obtained more decided pathogenic effects in guinea-pigs than Schenck seems to have done. These are differences that might reside just as well in the different races of animals used as in the organisms. In a personal communication Schenck states that on comparison the two organisms appear identical in form and in culture. Through the kindness of Dr. Schenck I was enabled to compare the two organisms and I could not detect any distinguishing differences either culturally or morphologically. The injection of Schenck's fungus into the abdominal cavity of a mouse (December 5, 1899), was followed by death after four weeks, and the anatomical and histological characteristics of the lesions did not differ in any essential from those of the lesions produced by the organism from Dr. Perkins' case.

It is regrettable that circumstances prevented the demonstration of the organism in the human lesions. More thorough study of the histological changes produced by the organism in man are also desirable. An abscess should be excised in an early stage of formation.

It is exceedingly interesting to note that we have here a pathogenic fungus that in the lesions it produces in animals exists in the spore-form, or in a modified spore-form, and that it undoubtedly multiplies as such; threads do not seem to develop in the tissues of susceptible animals. The exact manner in which the spores reproduce themselves under these circumstances I have not attempted to determine.

The fungus produces a slow, circumscribed and nodular inflammation with necrosis and pus formation in the centre and the development of granulation and fibrous tissue at the periphery—encapsulation. This is seen especially well in the abdominal cavity of white

rats and mice. The destruction of tissue after subcutaneous inoculation in mice may be quite extensive, large ulcerated surfaces with undermining and purulent infiltrations at the margins being the result. In less susceptible animals, such as the dog, dense areas of scar tissue are produced.

A characteristic clinical feature of the human cases is the refractory nature of the subcutaneous abscesses. This was pronounced in both Schenck's case and in the case under Dr. Perkins' care. Brayton[3] describes a similar case clinically; it occurred in a healthy, young florist who punctured a finger with a wire while making bouquets; a succession of chronic abscesses with gelatinous contents appeared, extending during a period of two months from the finger to the elbow, much scarring being left behind. This case was not examined bacteriologically.

The three cases have much in common; in all a succession of similar, refractory, small abscesses of the upper extremity developed consequent upon injury by similar means; in Schenck's case the scratch of the skin of the finger by a nail; in Perkins' case the blow upon the finger by a hammer; and in Brayton's case the puncture of a finger by a wire.

DESCRIPTION OF PLATES II AND III.

PLATE II.

Fig. 1—Photograph of arm of patient, showing ulcers and scars, at a late stage of the lesions.

Fig. 2.—An original culture of the sporothrix, three weeks old.

Fig. 3.—Slant culture on glucose agar, 4 days old.

Fig. 4.—Slant culture on glucose agar, 8 days old.

PLATE III.

Fig. 5.—Colonies on glycerine-agar plate, 48 hours old.

Fig. 6.—Margin of living hanging drop culture (gelatine). ×150.

Fig. 7.—Same as Fig. 6. ×1000. Unstained living culture.

Fig. 8.—Photograph of section of abdominal nodule in white rat. ×1000. Gram's stain. Cells and spores, the latter oblong and deeply colored.

[3] *Indianapolis Medical Journal*, 1899, xviii, p. 272.

FIG. 1.

FIG. 4.

FIG. 3.

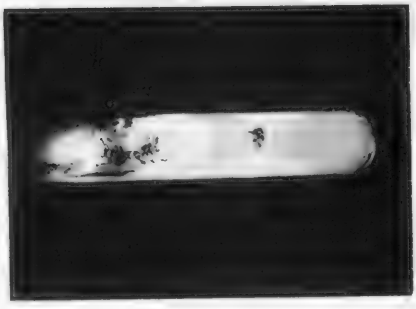

FIG. 2.

FIG. 5.

FIG. 7.

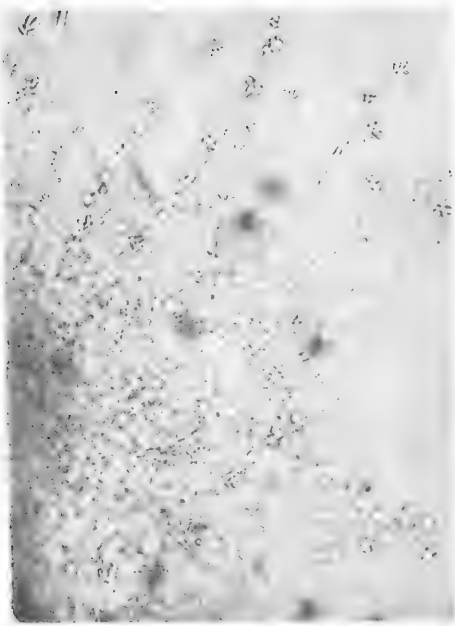

FIG. 6.

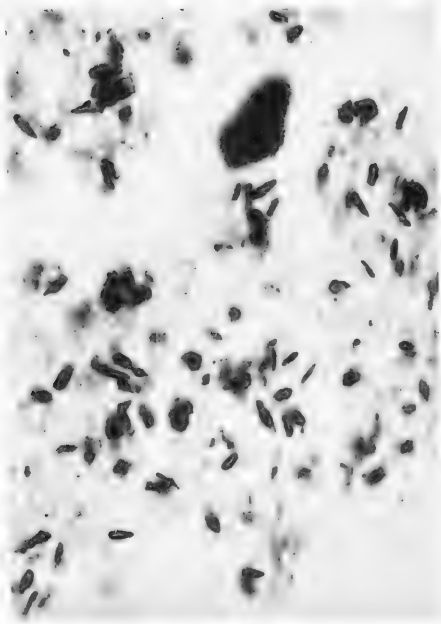

FIG. 8.

THE HELIOTYPE PRINTING CO., BOSTON.

PATHOLOGICAL REPORT ON A CASE OF DERMATITIS VESICULO-BULLOSA ET GANGRÆNOSA MUTILANS MANUUM (DUHRING), WITH A CONSIDERATION OF THE RELATIONS OF VASCULAR AND NERVOUS CHANGES TO SPONTANEOUS GANGRENE AND RAYNAUD'S DISEASE.[1]

By WILLIAM G. SPILLER, M. D.

(*From the William Pepper Clinical Laboratory, Phoebe A. Hearst Foundation.*)

PLATES IV AND V.

This case was reported clinically by Dr. L. A. Duhring[2] in 1892 under the title of a "Case of Dermatitis Vesiculosa Neurotraumatica of Forearm." Dr. Duhring thought that the case was obscure and difficult to classify, and notwithstanding the presence of hysteria he believed that the symptoms could possibly be explained by regarding the process as a traumatic ascending multiple neuritis, although he was guarded in expressing this view.

A later clinical history was published by Dr. Sinkler[3] in 1897, in which much of the earlier history given by Dr. Duhring is included. A brief abstract of Dr. Sinkler's paper is as follows:

The woman, A. A., was 35 years of age in 1897. For years she had had many symptoms of general nervous disorder which included frequent and protracted attacks of gagging and vomiting, palpitation of the heart, crying spells and globus hystericus. In Sept., 1890, she was burnt with a flat-iron on the flexor surface of the left forearm just above the wrist, the area being about the size of a silver dollar. The burn was superficial but did not heal readily or completely, and from some unknown cause began to break out anew. Within a month of the acci-

[1] Read before the Section on Neurology and Medical Jurisprudence of the American Medical Association, June 5, 1900.

[2] *International Medical Magazine*, 1892, i, p. 140.

[3] *Journal of Nervous and Mental Disease*, 1897, xxiv, p. 687.

dent it began to show a superficial gangrenous patch which remained about six weeks. The whole forearm became reddened and the seat of throbbing and darting pain. About six weeks after the accident the burn seemed to be nearly healed, and then a single pimple, a papulo-vesicle, formed on the extensor surface of the forearm near the burn. In a week or two this lesion ulcerated and crusted, and then other similar pimples formed near the original one; some of them vesicles and some blebs, covering by degrees the greater portion of the wrist. The morbid process continued on the left wrist and upper part of the hand for three years, migrating from place to place and breaking out anew as soon as any point became healed. By the early part of 1894 the left arm was entirely healed.

Just at the time of the healing of the left arm a papulo-vesicle similar to those which had invaded the *left* arm appeared at the end of the *right* index finger. This followed the same course of breaking out into an ulcer and then healing; new papulo-vesicles formed on this finger and then attacked the adjoining fingers. The affection subsequently spread to the dorsal and palmar surfaces of the hand and finally implicated all of the fingers. At times gangrenous patches appeared on the fingers, followed by sloughing and more or less loss of tissue. In this way the fingers and thumb were lost. The patient suffered from pain in the right hand. Objective sensation was unimpaired. The sloughing began with discoloration of the skin and the skin rapidly became black and dry and the slough was thrown off leaving a granulating surface. The urine was free from sugar.

Dr. Sinkler concluded after a careful study of the case that the disease was a trophoneurosis dependent upon an hysterical diathesis.

I made a brief examination of the patient in June, 1899, and observed certain symptoms that caused me to believe that the woman had Graves' disease. I made no notes of her condition, knowing that the case had been studied clinically by Dr. Duhring and Dr. Sinkler. Dr. Sinkler later informed me that the patient had marked evidences of Graves' disease; at least she had distinct exophthalmos and rapid heart's action without much, if any, thyroid enlargement.

In June, 1899, the right hand was amputated by Dr. W. J. Taylor just above the wrist and was given to me for examination (see Plate IV, Fig. 1). The part had become useless and pus had formed in the stump of the fingers. Dr. Sinkler tells me that the patient had

been for years an opium eater. After the amputation she did very well and was improving steadily when one day she received a large quantity of opium pills from some friends. She probably took several of these, as in the evening after the visit of her friends she vomited and was found in a condition of stupor. The next morning she had a convulsion. The urine was examined but no evidence of nephritis was obtained. The convulsions became more frequent, the stupor continued, and she died on the third or fourth day after taking the opium.

Previous to her death Dr. Duhring[4] published another clinical report of this patient and a beautiful colored drawing of the right hand.

Dr. C. W. Burr made the necropsy and kindly placed at my disposal the brain, cord and some of the peripheral nerves.

Dr. Joseph Walsh made a bacteriological examination of the amputated hand. Important results were not expected from this examination as the parts were exposed to various kinds of infection. His report is: "Aërobic and anaërobic cultures were made from suppurative foci on the fingers and hands and from non-suppurative portions. The results were positive and pure cultures of staphylococci were obtained, the great majority being white staphylococci. Inoculation into guinea-pigs of pus from the suppurating foci and of the pure cultures in agar produced no effect."

The microscopical examination of the tissue was made by me.

Right upper extremity: Ulnar nerve and artery.—The ulnar artery taken from the amputated limb is much thickened, both in the media and intima, especially in the latter. The lumen at one part is almost entirely filled by an organized thrombus (see Plate IV, Fig. 2). An elastic membrane is seen along one side of this thrombus. This thrombus is separated from the intima by a clear space throughout most of its extent.

The nerve fibres of the ulnar nerve in the same section in which the thrombosed ulnar artery is found, appear to be normal by ammonia carmine and the Weigert hæmatoxylin stains. The ulnar artery towards its peripheral terminations is still much thickened, and yet the nerve fibres of the accompanying nerve are normal by ammonia carmine or

[4] *International Atlas of Rare Skin Diseases*, xiv, March 22, 1899.

Weigert's hæmatoxylin stain, or at most are very slightly altered. A section of the ulnar nerve taken from the amputated limb and stained by Marchi's method shows only a moderate amount of black masses within the nerve fibres, and it is questionable whether the nerve could be regarded as degenerated from the evidence furnished by this method.

Nerve fibres from the right hand—I cannot say which nerve—taken just above the finger and stained in the fresh state by osmic acid and teased appear to be normal. The same is true of nerve fibres taken from the lower third of the right forearm.

Median nerve.—A piece of the median nerve, taken about 8 cm. above its termination in the stump, shows some proliferation of the endoneurium with possibly an atrophy of some of the nerve fibres. The extreme end of the branch to the middle finger is considerably degenerated and the endoneurium is much proliferated. Many nerve fibres have disappeared and the small accompanying arteries are much thickened. The extreme end of the branch to the index finger shows some degeneration of the nerve bundles. The extreme end of the branch to the thumb shows distinct degeneration of nerve fibres and proliferation of the endoneurium, but many nerve fibres are still present.

A section from the median nerve of the amputated hand and forearm stained by the Marchi method shows degeneration in some of the nerve fibres, but the degeneration is not excessive and is not so great as that seen in the left brachial plexus. Masses stained black by the osmic acid are seen in many of the fibres.

Radial nerve.—The radial nerve from the amputated part, taken 6 cm. above the styloid process of the radius, shows distinct diminution in the number of nerve fibres and overgrowth of the endoneurium (see Plate V, Fig. 3), but the degeneration is slight in comparison with that of the distal portion of the branch to the thumb. With the Marchi stain the degeneration 6 cm. above the styloid process is considerable. At the distal portion of the branch of the radial nerve to the thumb (Plate V, Fig. 4) the alteration of the nerve fibres is greater than in any other nerve. The fibres here have mostly disappeared and the connective tissue is greatly proliferated.

A branch of the radial artery taken just above the thumb and 4 cm. below the styloid process of the radius may be regarded as normal, although the radial artery examined 8 cm. above the styloid process shows distinct, but not excessive overgrowth of the intima, this being considerably less than that seen in the ulnar artery.

Right brachial plexus.—The brachial artery and the nerve fibres ap-

pear to be normal by Weigert's hæmatoxylin stain and the ammonia carmine, and the degeneration is unimportant by the Marchi method.

A vein taken from the back of the right hand shows some proliferation of the intima.

Left upper limb.—A small portion of the radial nerve and a portion of the brachial plexus were the only tissues of this extremity placed at my disposal for study.

The left radial nerve at a point unknown to me shows as distinct a diminution of nerve fibres and proliferation of the endoneurium as does the right radial 6 cm. above the styloid process.

The intima of the left brachial artery is slightly thickened. The nerve bundles of the plexus by the ammonia carmine stain are normal and yet the Marchi method shows a very intense degeneration of the myeline, which must have been recent—a greater degeneration indeed than is seen in any other nerve except perhaps the right radial. In the left brachial plexus in certain nerve bundles are a few areas in which no nerve fibres exist and only loose fibrous connective tissue is found. These areas are sharply defined from the surrounding nerve fibres of the bundle. In one bundle two such areas are seen. It is impossible to say whether these are imperfections in the original development of the tissue or the result of degeneration of nerve fibres.

Muscle.—The first interosseous muscle of the right hand is not much altered. The muscular fibres are nearly normal in size. The larger intramuscular nerve bundles are wonderfully well preserved, although the smaller bundles show some proliferation of the endoneurium. The intima and media of some of the small vessels within this muscle are thickened.

The skin from the back of the hand just above the fingers has lost the epidermis and the papillæ of the cutis vera are flattened.

Spinal cord.—Sections were taken for microscopical study from the lower cervical and upper thoracic regions and were found to be normal. Nissl's method could not be employed as the spinal cord had been put in Müller's fluid. Some of the motor cells of the anterior horns are vacuolated, but the cells have normal processes and do not appear to be atrophied. Cells found in the area corresponding to Clarke's columns are not atrophied, and so far as can be determined by the carmine stain they are normal. The nuclei in some are eccentric.

The medulla oblongata is normal. The brain by macroscopical examination is normal.

The lesions in this case in brief are as follows: The central nervous system is normal. The nerves in the distal portion of the right hand near the metacarpo-phalangeal articulations are much altered, especially the radial; the ulnar less distinctly so. The right median nerve is slightly altered at about 8 cm. above its terminal portion in the stump of the hand, and the right radial 6 cm. above the styloid process is somewhat degenerated. The nerves could not be studied at higher levels, as the tissue necessary for this was not in my possession. The right brachial plexus is normal. The ulnar nerve is normal except in its most distal portion where the alteration is slight.

The arteries of the right upper limb are diseased in some parts. The ulnar artery near the wrist shows the greatest amount of thickening of the intima and at one portion an organized thrombus is found. The right radial artery shows some proliferation of the intima.

The brachial plexus of the left side gives evidence by Marchi's method of intense recent degeneration, but this is not seen by the ammonia carmine stain. The intima of the left brachial artery is slightly thickened. The left radial nerve is not entirely normal.

It does not seem probable that the vascular changes alone could have produced the peculiar trophic lesions of the right upper limb. Even the organized thrombus of the right ulnar artery had not caused degeneration of the accompanying nerve, and the changes in the right radial artery were not very important. The slight alteration of the ulnar nerve in its most distal portion was probably the result—as was the alteration in the other nerves of the hand—of spontaneous amputation and gangrene, and not of thrombosis of the ulnar artery. The veins had not escaped. The nerves of the right upper limb were seriously diseased only at their terminations near the gangrenous area, except perhaps the radial which was diseased at least 6 cm. above the styloid process of the radius. It is questionable whether so extensive lesions can be explained by so slight degeneration of nerves.

Where peripheral gangrene occurs and the vessels and nerves of the gangrenous limb are found diseased several questions are at once suggested:

1. Was the gangrene caused by the endarteritis obliterans?

2. Was the gangrene caused by the degenerative changes in the nerves?

3. Was the degeneration of nerves and vessels the result of the gangrene?

4. Were the nerve lesions the result of vascular disease?

5. Was the degeneration of the vessels produced by the changes in the nerves?

These questions can be best answered by a study of the cases reported in literature, and they will be considered seriatim.

Raynaud[5] began one of his papers on symmetrical gangrene with a quotation from a work of Victor François written in 1832: "Everything concerning spontaneous gangrene is in a state of distressing uncertainty." These words are not quite so true as they were at the time they were first written, but much of this "distressing uncertainty" still remains. Cases of gangrene in which the cause cannot be determined are still reported as shown by an interesting paper by McFarland.[6]

1. *Can gangrene be caused by endarteritis obliterans?*

Many cases have been reported in which obliterating endarteritis was believed to be the cause of gangrene. The description of the "gangræna ex endarteriitide hyperplastica" as given by Billroth represents the disease as beginning with prodromal symptoms lasting many years, viz., disturbance of circulation, cyanosis of the limbs, a sensation of cold and weight, paræsthesia, and inability to stand long or walk far. The gangrene is produced by a slight cause and is usually moist.

Von Winiwarter[7] concluded from his examination of several cases of so-called primary, spontaneous gangrene that the underlying cause is an endarteritis terminating in complete closure of the affected vessels. Zoege von Manteuffel[8] finds that this form of endarteritis is the result of successive deposition and subsequent organization of layers of thrombi,

[5] "On Local Asphyxia and Symmetrical Gangrene of the Extremities," by Maurice Raynaud. Translated by Thomas Barlow. London, 1888. The New Sydenham Society, vol. cxxi.

[6] *Transactions of the College of Physicians of Philadelphia*, 1898, 3. s., xx, p. 160.

[7] *Arch. f. klin. Chirurg.*, 1878, xxiii.

[8] *Deutsche Zeitschr. f. Chir.*, 1898, xlvii, p. 461.

so that finally the lumen of the vessel becomes filled with vascularized connective tissue, and Hoegerstedt and Nemser[9] believe that in general thrombosis participates in a similar way in the production of obliterative endarteritis. Haga[10] in his interesting paper on spontaneous gangrene describes and pictures obliterative endarteritis, which he believes to be of syphilitic origin, as a cause of this disease. The association of gangrene with the group of symptoms called "intermittent claudication," studied by Charcot, Goldflam, Erb, and others, and shown to be dependent on arterial disease, is well recognized. It is not to be doubted, therefore, that gangrene may be caused by endarteritis obliterans, nor is it difficult to understand why this should be so.

Arterial thrombosis, with or without pre-existing arterial disease, is a demonstrated cause of senile gangrene, and may be the cause of the gangrene occurring occasionally as a complication or sequel of infectious diseases, particularly in influenza, typhoid and typhus fevers.[11]

2. *Can gangrene be caused by degenerative changes in nerves when the blood vessels are healthy?*

The idea that gangrene may be due to diseases of the nervous system alone without any vascular disease is not new. Raynaud[12] refers to the thesis by Zambaco[13] in which this view was expressed. The paper of Pitres and Vaillard[14] is often quoted in support of the possibility of gangrene resulting from degeneration of nerves. These writers reported two cases in which symmetrical gangrene of the feet occurred, and the arteries and veins were normal. The nerves of the lower limbs were much diseased below the knees, but not above. Those of the upper limbs were normal. Dehio[15] in criticising this paper states that the writers have not proven that gangrene may result from neuritis without vascular disease. Dehio, I think, is quite right in this criticism. The case does seem to show that endarteritis may be absent in gangrene, but the finding of nerve lesions and gangrene in the same limb does not prove that the latter is the result of the former. Both conditions may

[9] *Ztschr. f. klin. Med.*, 1896, xxxi, p. 130.

[10] Virchow's *Archiv*, 1898, clii, p. 26. Since the completion of this article C. Sternberg's paper (*Virchow's Archiv*, 1900, clxi, p. 199) has appeared with a full consideration of the relation of obliterating endarteritis to spontaneous gangrene.

[11] See Welch, Article "Thrombosis and Embolism" in Allbutt's System of Medicine, vol. vi, p. 178. London and New York, 1899.

[12] Loc. cit.

[13] Paris, 1857.

[14] *Arch. de phys. norm. et path.*, 1885, 3. s., v, p. 106.

[15] *Deutsche Zeitschr. f. Nervenheilk.*, 1893–4, iv, p. 1.

result from a common cause, or the gangrene may produce the degeneration of the nerves, as will be mentioned presently. In the report of a necropsy in a case of symmetrical gangrene Raynaud[16] says that the results obtained by himself were absolutely nil in so far as the circulatory system was concerned, so that without Pitres and Vaillard's case we have known for many years that gangrene may occur when the vessels are normal.

Dejerine and Leloir[17] in reporting two observations of gangrenous eschars of the skin, in which they found the nerves diseased, have collected most of the evidence existing at the date of their publication in favor of the occurrence of gangrene as the result of disease of the nervous system. The possibility that neuritis may produce gangrene must, I think, be admitted, although there is not agreement of opinion among authorities as to the interpretation of the experimental and clinical data adduced in support of this view.

3. *Does gangrene cause alterations of vessels and nerves?*

Lapinsky[18] has recently discussed this question quite fully and I cannot do better than to refer to his papers. The investigations of Hodson, Friedländer, Cornil and Ranvier, Ivanowski, Ziegler and others have shown that chronic inflammation has an injurious effect upon the vessels in the neighborhood and causes peri- and end-arteritis. Lapinsky noticed these changes of the vessels in some cases of his own in and near the gangrenous areas.

In reference to the nerves he says that the importance of local gangrene and suppuration in the production of changes in the nerves of the diseased limb has often been discussed without great weight being attributed to those conditions as etiological factors. He quotes a number of cases in which gangrene was found and was not believed to have caused degeneration of nerves, and he attributes no importance to the local gangrene in the production of the degeneration of the nerve stems in cases of his own. It is well known that ascending neuritis from a suppurating wound is of very rare occurrence.

Pitres and Vaillard allude to the fact that nerves passing to a gangrenous area do not necessarily show degeneration, and they say that this was recognized by Vulpian in 1866 and later by Dejerine and Leloir. However, it is probable that in some cases gangrene does cause alteration of nerves, possibly through alteration of the blood-vessels.

[16] Loc. cit.

[17] *Arch. de phys. norm. et path.*, 1881, 2. s., viii, pp. 989 and 391.

[18] *Deutsche Zeitschr. f. Nervenheilk.*, xv, p. 364.

4. *Does degeneration of blood-vessels produce changes in the nerves of the same territory?*

In studying nerves for degenerative changes it is not sufficient to examine sections taken at a distance from the peripheral ends of the nerves. The increase in the degree of degeneration of nerve fibres towards the periphery was observed by Mannkopf in 1878 in a case of embolism of the popliteal and crural arteries.[19] The greater alteration of peripheral ends of nerves has also been seen by Hans Gudden[20] and other investigators. The importance of the recognition of this fact is seen, for example, in a case of spontaneous gangrene reported by C. Sternberg.[21] The sciatic nerve of the diseased limb did not contain degenerated nerve fibres but the vessels were much altered. I am unable to determine from the report of this case whether or not the peripheral ends of the nerves in the amputated limb were studied.

Schlesinger[22] says that the primary nature of the vascular disease and the secondary nature of the neuritis are not recognized by all, but he thinks that the vascular alteration occurs first. He reports a case in which pain in the feet and livid discoloration of the feet and hands were followed after some months by gangrene of the left foot. The left foot was amputated and the arteries and veins of the nerves within it were much thickened. The nerve fibres were normal in many bundles but in most they were more or less altered and the connective tissue of the nerves was proliferated. He thinks that without doubt the vascular degeneration occurred before the degeneration of the nerve fibres in this case, and he seems to have based this opinion chiefly on the clinical signs, and yet the disease began with pain in the feet as well as with livid discoloration.

When closure of an artery occurs the degeneration of the nerves may be only in the part below the thrombus, as seen in cases studied by Lapinsky. It is not necessary to quote many examples of this. We can accept without dispute the statement that a nerve speedily degenerates when its blood supply is abruptly cut off. Lapinsky says that in cases of acute ischæmia he found the changes of the nerve fibres more marked towards the distal ends where the effects of closure of the arteries were most felt.

The nerve changes are not so perceptible in chronic vascular disease,

[19] Cited from Lapinsky.

[20] *Arch. f. Psychiatrie*, 1896, xxviii, p. 643.

[21] *Wiener klin. Wochenschr.*, 1895, viii, pp. 650, 687.

[22] *Neurologisches Centralblatt*, 1895, xiv, pp. 578, 634.

and according to Lapinsky they have been seen in only comparatively few cases. In some cases the changes of the nerve fibres were very slight and occurred only in certain areas; in other cases the nerve fibres were well preserved and the connective tissue about them was proliferated; in still other cases the nerves were perfectly normal. Lapinsky has collected the reports of a number of cases from the records bearing on this subject. He observed 8 cases of vascular disease; in 7 of these the arteries of one lower extremity were affected, and in one the arteries of both extremities were diseased, and gangrene developed in the part imperfectly nourished. The connective tissue of the nerves was increased in all the cases and this was especially true of the endoneurium.

In the case reported in the present paper the ulnar nerve showed no degeneration as a result of the thrombosis of the ulnar artery.

Joffroy and Achard[23] seem to have been the first to describe neuritis of vascular origin. In a case of neuritis they found that the most pronounced lesions of the vessels were associated with the most pronounced lesions of the nerves, and from this they concluded that the degeneration of the nerves was due to the thickening and obliteration of their nourishing arteries. Neither this case nor the one published by Dutil and Lamy[24] establishes beyond question the vascular origin of neuritis although such an origin seems very probable. Dutil and Lamy say that in their case the parallelism existing between the vascular and nervous lesions justifies attributing the degeneration of the nerves to the thickening and obliteration of their nourishing arteries.

5. *Does degeneration of nerves cause alteration of the vessels in the same territory?*

Bervoets[25] claims to have demonstrated that cutting nerves causes thickening of arteries in the same territory, and he believes that he has demonstrated that neuritis is a cause of endarteritis. A. Fraenkel[25a] obtained similar experimental results, whereas C. Sternberg[25b] had only negative results. Czyhlarz and Helbing[25c] find an explanation of this discrepancy in their observation that changes in the vessels following experimental lesions of nerves occur only when ulcers result from the operation. Lapinsky[26] has collected from the records a large amount

[23] *Arch. de méd. expér.*, 1889, i, p. 229.
[24] *Arch. de méd. expér.*, 1893, v, p. 102.
[25] Over spontaan gangreen, etc., Nykerk, 1894.
[25a] *Wiener klin. Woch.*, 1896, ix, pp. 147, 170.
[25b] Loc. cit.
[25c] *Centralbl. f. allg. Path. u. path. Anat.*, 1897, viii, p. 849.
[26] *Zeitschr. f. klin. Med.*, 1899, xxxviii, p. 223.

of evidence in support of the neuritic origin of endarteritis, and he concludes that these vascular disturbances may be of several varieties. In some cases the lumen is enlarged in the vessels of the territory in which the diseased nerves lie, and this part of the body becomes hyperæmic and its temperature is raised. The vessels may become broadened and lengthened and tortuous. In some cases the nutrition of the vascular walls is affected, as shown by local œdema and occasionally small hæmorrhages in the distribution of the diseased nerves. In some cases anatomical changes in the vessels have been found.

Lapinsky refers to a number of clinical cases in which œdema or redness and increase of temperature followed injury or disease of nerves. This causes us to think of erythromelalgia, inasmuch as this redness and increase of temperature were found in later stages of neuritis as well as in the early. Alteration of nerves and vessels was very marked in a case of erythromelalgia reported by Dr. S. Weir Mitchell and myself.[27] Lapinsky refers to a number of cases in which changes in the walls of the vessels were believed to result from neuritis. He reports two cases in which disease of the walls of the vessels developed in the distribution of diseased nerves. He believes that the disease of the nerves causes a loss of tonicity and elasticity in the walls of the vessels and a disturbance in the nutrition of the vessels; the enlargement of the lumen, the increased intravascular pressure and the slowing of the blood current lead to further changes.

I have referred elsewhere[28] to the views of Thoma. Thoma has shown that when the lumen becomes too great in proportion to the amount of blood flowing through it, as for example after amputation, a compensatory connective tissue thickening of the intima occurs and the proper relations are restored. He has shown that neuritis produces a similar change in the vessels.[29] He studied the soft tissues taken from both temples in a case of left supraorbital neuralgia. More or less hyperæmia occurred in the painful area at the time of the attack. He found that the arteriosclerosis in the area of the supraorbital neuralgia was considerably greater than in the corresponding area on the other side. Thoma had acquired so extensive a knowledge of the vascular system that he was able to name most of the large arteries when transverse microscopical sections of them were shown to him. It seems that the vasomotor change caused by the pain in Thoma's case led to this thickening of the intima.

[27] *Amer. Jour. Med. Sciences*, Jan., 1899, cxvii, p. 1.

[28] Ibid.

[29] *Deutsches Arch. f. klin. Med.*, 1888, xliii, p. 409.

More recently this subject has been again studied by Lapinsky.[30]

Although the evidence, especially on the experimental side, is conflicting, there is support for the view that degeneration of nerves may cause degeneration of vessels.

From the preceding statements and review of the evidence relating to the association and correlation of arteritis, neuritis and gangrene it may, I think, be stated:

1. Gangrene may be caused by endarteritis obliterans.

2. Alteration of nerves alone without alteration of the vessels is believed by some to be a cause of gangrene. We need probably more evidence before this conclusion can be definitely accepted.

3. Gangrene may cause degeneration of the vessels, especially of the portions near the gangrenous area.

4. Gangrene is less liable to cause degeneration of nerves except of the portions within or near the gangrenous areas.

5. Sudden closure of blood-vessels causes degeneration of the nerves nourished by these vessels, unless an adequate collateral circulation is promptly established. If the vascular disease is of a chronic type the nerves may escape, at least for a time, but do not always do so, the result doubtless depending upon circulatory conditions which vary in different cases.

6. Degeneration of nerves is a possible, but not thoroughly demonstrated, cause of degeneration of the blood-vessels.

I have noticed in this and other cases of arterio-sclerosis a multiplication of the elastic membrane. This is probably a new formation. It has been regarded by some as merely a separation of the layers of the previously existing elastic membrane, but this explanation is not satisfactory for all cases. In order to furnish so much new elastic tissue the old must have become very much thickened. I have found an elastic membrane in the organized thrombus of the case A. A., and the thrombus was here separated by a clear space from the proliferated intima. It probably represents here newly-formed tissue. Similar views are held by Dmitrijeff[31] and others.

[30] Lapinsky, *Arch. de méd. expér.*, 1899, xi, p. 109.

[31] *Ziegler's Beiträge*, 1897, xxii, p. 207.

The case of A. A. was not a typical one of Raynaud's disease but it bore certain resemblances to it as Dr. Sinkler pointed out. Local syncope or local asphyxia is not mentioned in the history. The affection was of the distal parts of both upper extremities, in which papulovesicles were important features. Dr. Sinkler describes the sloughing process as first a discoloration of the skin, involving perhaps one-half the surface of the finger; this portion of the finger rapidly became black, then dry, and the slough was thrown off leaving a granulating surface. This account is not unlike that of a case of Raynaud's disease. Monro,[32] in his excellent monograph on Raynaud's disease, says that in less than two per cent of the cases of this disease gangrene alone is mentioned, and a careful perusal of these cases makes it almost certain that there was a stage of asphyxia. In the same proportion of cases, syncope and gangrene alone are mentioned, but in the majority of these asphyxia also was probable. This case A. A. could be considered at most only as an atypical one of Raynaud's disease, but it may have a similar etiology.

There is a class of trophic diseases having certain resemblances to one another but still with distinctive features. Erb[33] compares Raynaud's disease with intermittent claudication. The two disorders resemble one another in the paræsthesia and pain, in the vasomotor disturbances, in the cutaneous gangrene of the fingers and toes, and in the occasional mutilation. In Raynaud's disease neurasthenia, even psychopathies, may occur; the fingers are chiefly implicated; the gangrene is usually limited to the superficial layers of the skin; the symptoms are paroxysmal but not so intermittent as in intermittent claudication and do not depend directly on the use of the limbs. In Raynaud's disease marked changes in the vessels (arteriosclerosis, absence of pulse) and severe gangrene have not been observed. After making these distinctions, Erb refers to Dehio's findings of endarteritis and phlebitis in a case of Raynaud's disease, and says that after all there may be a closer resemblance between Raynaud's disease and intermittent claudication than has been supposed.

[32] T. K. Monro, Raynaud's Disease. 1899.

[33] *Deutsche Zeitschr. f. Nervenheilk.*, 1898, xiii, p. 1.

Angiosclerosis, according to Erb, is manifested clinically in a variety of forms. Simple or obliterating arteriosclerosis without nervous symptoms causes senile gangrene or simple spontaneous gangrene; the combination of obliterating arteritis with symptoms of vasomotor irritation and of sensory and motor disturbance causes the intermittent lameness; the combination of symptoms of vasomotor paralysis (possibly of irritation of the vasodilators) with sensory irritation and obliterating arteritis is possibly the cause of erythromelalgia; the combination of obliterating arteritis with vasomotor and trophic and nervous symptoms may be the cause of Raynaud's disease; the combination of arteritis with intense degeneration and inflammation of the nerves causes the angiosclerotic neuritis of Joffroy and Achard, Dutil and Lamy, and Schlesinger; the vasomotor and sensory irritation without the endarteritis causes acroparæsthesia. It is to be noted that in these diseases a functional disturbance in addition to an organic one is common, and in reading the clinical report of the case A. A. as given by Dr. Duhring and Dr. Sinkler I am impressed by the fact that the disturbance was certainly in large part functional.

The interpretation of the lesions found in the case A. A. is difficult. The woman had stigmata of hysteria and probably had Graves' disease. Destruction of nerves near the seat of spontaneous amputation is nothing more than one might expect as a result of the amputation and suppuration. This degeneration decreased upward quite rapidly, and 6 cm. above the styloid process of the radius was slight in the radial nerve except by the Marchi method and was not very evident in the ulnar or median nerve at parts only a short distance from the seat of spontaneous amputation. The vascular disease seems hardly sufficient to explain the loss of the fingers. The ulnar artery from the amputated limb was nearly closed at one part by an organized thrombus, but this had not caused degeneration of the accompanying ulnar nerve, and it is hardly reasonable to suppose that it had caused such serious changes as the loss of the fingers. The alteration of the radial artery was certainly insufficient to explain the symptoms. The right brachial plexus was normal and there was no evidence here of ascending neu-

ritis. The alteration of the left brachial plexus as seen by the Marchi method is difficult to explain. The appearances were those of recent degeneration. Shall we believe that trophic lesions would have reappeared in the left upper limb if the patient had lived longer, or shall we believe that the findings were artefacts? The left radial nerve was as much altered as the right radial 6 cm. above the styloid process of the radius. This alteration may have been due to the serious lesions that had formerly existed in the left upper limb. Dr. Sinkler regarded the case as a trophoneurosis dependent upon an hysterical diathesis. There is much to be said in favor of this opinion, but there was nevertheless neuritis of high degree in the periphery of the right upper limb, moderate degeneration of nerves several centimetres above the wrist of the same limb, and some vascular change in the amputated part; and the radial of the left upper limb was diseased. The question as to whether these lesions were primary or secondary I do not think can be positively determined. I have attempted to show that they might have been either. At all events the condition in A. A. was not the result of any distinct lesion in the central nervous system. Functional disturbances in the circulation of the peripheral ends of the limbs, in connection with the organic changes, probably increased the peculiar lesions in the case A. A. Her relapses seemed to depend to some extent on functional disorder.

Dehio [34] examined fingers that had been amputated on account of Raynaud's disease and found in these endarteritis, endophlebitis and degenerated nerves, but he only had about 1 cm. of normal tissue above the gangrenous area for his investigation. He, too, was uncertain whether the vascular sclerosis preceded the gangrene or vice versa.

The microscopical examination in the case I report is valuable on account of the extreme rarity of a case of this character with necropsy. It would be most unscientific, however, to be too positive in the interpretation of the lesions, although these were of a definite character, as I have already shown that there is at present considerable difference of opinion concerning the explanation of similar or identical lesions in cases of gangrene.

[34] *Deutsche Zeitschr. f. Nervenheilk.*, 1893–4, iv, p. 1.

Dr. Duhring in his two papers in which he reports clinically the case A. A. refers to several similar cases in the literature. A brief review of some of these may be of interest and of assistance in judging of the etiological value of the findings in the case A. A.

Doutrelepont's [35] case was one of multiple gangrene of the skin over a large portion of the body, associated with vesicles and following the penetration of a needle beneath the left thumb in a hysterical woman. The spinal cord and nerves examined in this case were found to be normal, and the results of the necropsy did not in any way explain the disease.

The case reported by Kopp [36] resembles that reported by Doutrelepont. In Kopp's case the lesions were observed on the left breast, left forearm and left thigh. The neurotic nature of the affection Kopp believed was shown by the unilaterality, the acute development of the lesions in groups, the typical course, and the accompanying neuralgia. An ulcerating keloid on the left hand which developed in the scar of a burn, he thought might have caused ascending neuritis and implication of the spinal cord. Kopp describes the case as one of multiple neurotic cutaneous gangrene.

Galton's [37] patient was a girl of seventeen who had suffered from fits of an epileptic nature, brought on by a fright at school. Patches of redness followed by blebs appeared on the left wrist, hand and arm shortly after she had chopped off the distal phalanges of the index and ring fingers and cut through the middle phalanx of the middle finger. The eruption was peculiar from the rapid way in which it spread. Sometimes within a quarter of an hour the whole hand and arm would be covered with large blebs which would burst and discharge. The circulation seemed very feeble. At one time a crop of vesicles appeared on the left leg. Galton attributed the lesions to a reflex irritation.

Kaposi's [38] patient was a girl of twenty-two years, who had injured her right middle finger by a nail. The part was bound in iodoform. A few days later vesicles appeared on the dorsal surface of this finger and extended and affected the back of the hand and forearm. These vesicles were accompanied by a sensation of burning in the part. Other cutaneous surfaces of the body were attacked. Kaposi did not believe a

[35] *Vierteljahresschrift f. Dermat. u. Syphilis*, 1886, xiii, p. 179, and *Arch. f. Dermat. u. Syphilis*, 1890, xxii, p. 385.

[36] *Münch. med. Wochenschr.*, 1886, p. 665.

[37] *British Med. Journal*, 1891, i, p. 1282.

[38] *Wiener klin. Wochenschr.*, 1890, p. 425.

neuritis existed but thought that an ascending irritation was present and caused redness, exudation and vesicles, and that this irritation extended to the spinal cord. He thinks his case resembled Doutrelepont's, and that it was an expression of hysterical irritability of the vasomotor system, analogous to cases of herpes zoster gangrænosus and of zoster gangrænosus hystericus. No gangrene was observed in Kaposi's case. He calls his case "pemphigus neuroticus hystericus."

Bayet [39] describes a condition known as disseminated cutaneous gangrene. He speaks of it as a very rare affection. Some of the cases have been described, he says, as gangrenous zona, others as pemphigus neuroticus, and others as gangrenous urticaria. The causes of this confusion are the rarity of the affection and the predominance of certain symptoms in different cases; but common to all are the dependence of the lesions on disturbances of innervation and the local evidences of hysteria. Bayet's case is as follows:

A hysterical male, nineteen years of age, burned himself superficially on the anterior surface of the left forearm a little above the wrist. The wound healed at the end of twelve days. Two days after the accident plaques covered with a dry crust appeared on the external surface of the thumb. This crust did not last very long and left a superficial ulcer requiring two months to cicatrize. Within a short time twenty-one ulcers of different sizes, some as large as a franc, appeared on the left forearm. All these ulcers were found on portions of the skin which had not been in contact with the sulphuric acid. These lesions were found later on the hand. Deeply pigmented areas represented the former site of ulcers, and in some of these areas bullæ, containing a sero-sanguineous fluid, appeared. The skin between these lesions seemed to be normal. The case was believed by Bayet to be a multiple gangrene of the skin dependent upon hysteria. He was able to produce a characteristic lesion by suggestion.

Janovsky and Mourek [40] in a study of multiple cutaneous gangrene report two cases in which vesicles were observed but no necropsy was obtained. They give references to several cases of cutaneous gangrene. Whether these cases of cutaneous gangrene should be classed with such a case as that of A. A. in whom the lesions were chiefly of the character of vesicles is questionable. The etiology in all is obscure.

[39] *Annales de dermat. et de syphiligraphie*, 1894, v, p. 501.

[40] *Arch. f. Dermat u. Syphilis*, 1896, xxxv, p. 559.

DESCRIPTION OF PLATES IV AND V.

PLATE IV.

Fig. 1.—Photograph of the amputated part of the right forearm and hand, palmar aspect.

Fig. 2.—An organized thrombus in the right ulnar artery from the amputated part of the limb.

PLATE V.

Fig. 3.—Section of the radial nerve from the right forearm taken 6 ctm. above the styloid process of the radius. The nerve fibres are diminished in number and the endoneurium is proliferated. The degeneration is much less than in the more peripheral portion of the nerve (see Fig. 4).

Fig. 4.—The radial nerve from the right upper limb in its terminal portion. The degeneration of nerve fibres and the overgrowth of connective tissue are extreme.

FIG 1.

FIG. 2.

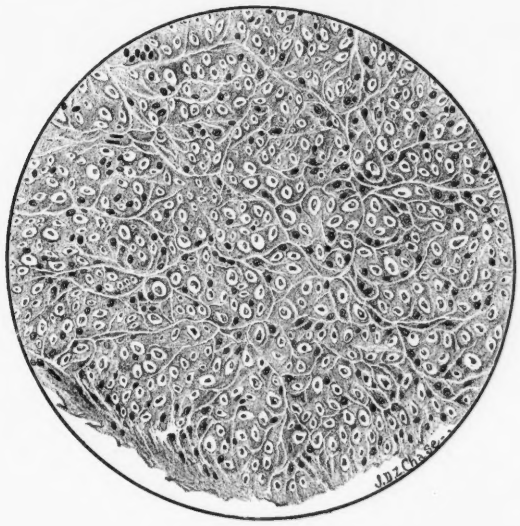

FIG. 3.

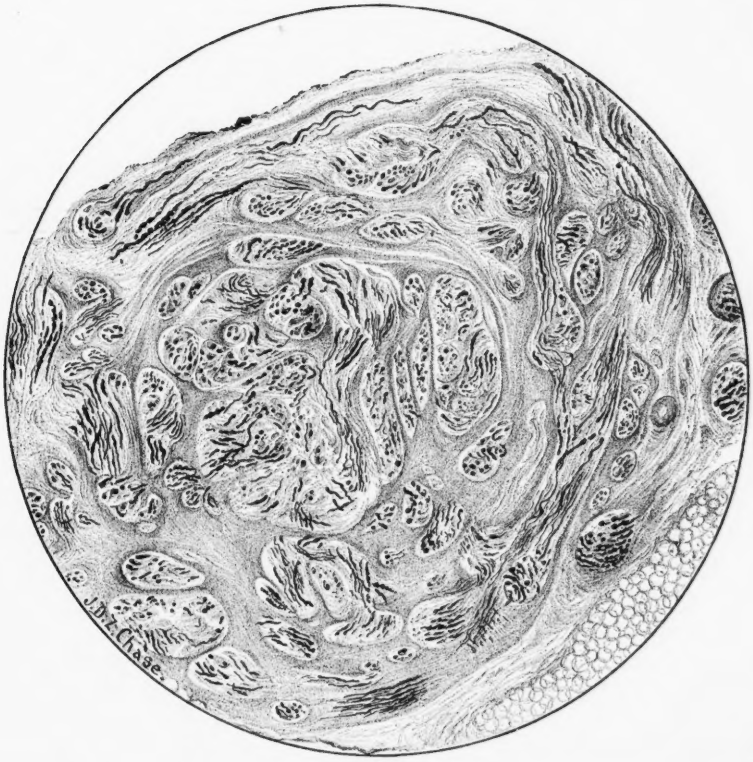

FIG. 4.